ABSTRACTS OF PRESENTATIONS MADE AT THE
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SMOKE SCIENCE AND PRODUCT TECHNOLOGY

(in alphabetical order of first authors)

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT39

Generation and assessment of whole cigarette smoke to *in vitro* systems - British American Tobacco's experience.

There are a number of exposure systems and devices that generate, dilute and deliver cigarette smoke for *in vitro* cell culture investigations. One such system used at British American Tobacco (BAT), Group Research & Development, Southampton, is the Borgwaldt RM20S smoke machine that delivers cigarette smoke to BAT's designed and developed whole smoke exposure chamber (patent publication number: WO 03/100417 A1). We have studied the effects of whole cigarette smoke on endpoints considered to be relevant to major smoking related diseases, namely cancer and chronic obstructive lung disease (chronic bronchitis and emphysema) including cell viability, oxidative DNA damage, gene expression and protein production of putative disease-related mediators. This system was first set up in 2003 and enabled the delivery of up to four different doses of cigarette smoke to cellular cultures at the air-liquid interface (ALI). This system has been recently expanded with the addition of another four syringe-unit to allow the generation and simultaneous delivery of eight different doses of cigarette smoke for *in vitro* analysis.

We have characterised the expanded whole smoke system by measuring smoke losses along the system, assessing precision of syringe delivery and by assessing smoke deposition within the chamber. Using a methane gas standard, syringe precision resulted in a repeatability error of ≤8%. An electrical mobility spectrometer measured smoke particulates across the system and results indicate ~60% of particulates reached the chamber and >22% deposited in the chamber. Assessment of delivery of whole smoke to six individual Transwells™ within the chamber using Neutral Red uptake assays indicated uniform delivery within the chamber.

Current and future whole smoke studies will investigate the biological effects of the vapour phase and toxicological effects of smoke generated from different smoke regimes including Canadian Intense. Such studies will utilise the capabilities of the Vitrocell Systems VC10 smoking machine.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SS06

Evaluation of a simple system for the measurement of smoke deposition in volunteers.

Historically studies on the deposition of tobacco smoke particulate matter have been based on measurements of inhaled and exhaled solanesol, an involatile long-chain alcohol specific to tobacco. This study examined the utility of determining deposition by measuring the optical tar, a measurement of the mass of tobacco smoke based on light obscuration, of puffed smoke and exhaled smoke. Two volunteer adult regular smokers (one male; one female) smoked a commercial 7 mg ISO tar yield King-Size cigarette in triplicate. Inhaled optical tar was measured by a smoking analyzer (SA7), respiratory behaviour and exhaled optical tar was measured using a prototype device called a BIBO. Smoke was exhaled through the BIBO and into an exhaled smoke sampling system for analysis by a particle spectrometer (Differential Mobility Spectrometer, Cambustion UK) and filter pad collection for off line analysis for solanesol. The aerosol properties of the inhaled smoke were characterized by

reproducing the SA7 puffing profiles on a Smoking Cycle Simulator for analysis by DMS. Inhaled levels of solanesol were estimated by filter analysis.

Deposition fraction was calculated for solanesol, DMS mass (assuming unit density of smoke particles) and optical tar. Deposition fractions based on optical tar and DMS data were in reasonable agreement. Solanesol measurements of exhaled smoke correlated well with optical tar and DMS mass measurements. Solanesol estimates of inhaled smoke from filter analysis did not correlate well with optical tar or DMS mass measurements. The data suggest that optical tar could be a convenient way of estimating smoke deposition fraction in the airways. However, further measurements should be taken with more volunteers. Additionally, the time resolution of the optical tar measurements of exhaled smoke may offer a way of determining the deposition of smoke in different anatomical regions of the lungs when combined with suitable deposition models.

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Migration of vascular endothelial cells *in vitro*: A cardiovascular disease model for RTP assessment.

A step in the formation of atherosclerotic lesions, the hallmark of cardiovascular disease, is damage to the vascular endothelium. Such damage may be enhanced by a pro-atherogenic inhibition of endothelial repair processes such as migration and proliferation. Cigarette smoking is a risk factor for cardiovascular disease, and it is thought that impaired endothelial repair processes in smokers contributes to increased likelihood of atherosclerotic lesion formation. Here, we describe the development of an endothelial damage repair assay in which we monitor the migration of vascular endothelial cells *in vitro* using a scratch wound assay.

Methods: Confluent monolayers of human umbilical vein endothelial cells (HUVEC) were scratched using a pipette tip. Migration of HUVEC across the wound was assessed over a period of 20 hours by real-time image capture. Analysis of wound width was performed using the IncuCyte platform (Essen Instruments). Cigarette smoke total particulate matter (TPM) from University of Kentucky 3R4F reference cigarettes was trapped on a Cambridge filter pad, eluted in DMSO, and added to cells immediately after wounding.

Results: When added to cells immediately after wounding, TPM inhibited endothelial migration in a concentration-dependent manner. We examined the reproducibility, repeatability and user variability of migration responses, all of which were within acceptable limits and with no statistically-significant variances in the effects of TPM. We also examined the effects of TPM obtained from reduced toxicant prototype (RTP) cigarettes compared to that obtained from appropriately-matched commercial control cigarettes. Statistical analyses showed that when using TPM derived from RTP cigarettes, inhibition of endothelial migration was less apparent compared to TPM derived from control cigarettes.

Conclusion: Migration of endothelial cells *in vitro* is a relevant cardiovascular system damage repair assay which demonstrates acceptable levels of data variability and is suitable for the assessment of the altered biological effects of RTP particulate phase smoke extracts.

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The role of carbon structure on cigarette smoke vapour phase toxicant reduction.

Cigarette smoke is a complex non-equilibrated mixture of chemicals (predominantly air) with thousands of compounds being generated from the incomplete combustion of tobacco. These are distributed between the gas phase and the particles (the particulate phase) that constitute the smoke aerosol. Activated carbon is a good filter material for the adsorption of many smoke vapour phase compounds, despite the challenging environment (complex mixture of chemicals, short contact times, filter format constraints etc). The carbon characteristics are important to maximise the adsorption, with a combination of macro/mesopores and micropores giving the greatest reductions. Experimental data showing how various carbons perform in a cigarette filter and how the data correlates with theoretical models will be presented. The focus will be on the adsorption of the smoke toxicants formaldehyde, acetaldehyde, acrolein, benzene and 1,3-butadiene. Emphasis will also be placed on the adsorption kinetics.

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***In vitro* model development at British American Tobacco.**

Traditionally, the biological effects of cigarette smoke have been tested by exposing cells in culture solely to the particulate phase of cigarette smoke in the form of total particulate matter (TPM). However, this approach does not facilitate investigation into the effects of gaseous and water-soluble components of cigarette smoke. Therefore, extensive efforts have been undertaken at British American Tobacco (BAT) to develop and test methods for exposing cells to whole smoke. This provides a physiologically more relevant means of testing the effects of smoke, in particular in *in vitro* models of cancer and chronic obstructive pulmonary disease (COPD). In addition, we are evaluating the generation and utility of aqueous cigarette smoke extract (CSE) as an exposure agent for our cardiovascular disease (CVD) models. Apart from models mimicking fundamental processes of the major smoking-related diseases, a number of assays to assess inflammation and oxidative stress endpoints have been developed and tested with different smoke exposure agents. We propose that results from these models and assays will provide sufficient scientific evidence to form the basis of a weight of evidence approach that will aid in the risk assessment of future reduced toxicant prototype products. This presentation will discuss the development of exposure systems and *in vitro* models at BAT, their utility in product assessment and their strengths and limitations.

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Cardiovascular disease biomarkers study. Part II: Tobacco-related biomarkers of exposure in exclusive cigarette smokers, exclusive moist snuff consumers, and non-consumers of tobacco.

A CVD biomarkers study conducted in the US measured putative tobacco-related biomarkers in three exclusive cohorts: cigarette smokers, moist snuff consumers and non-consumers of tobacco. Subjects were generally healthy males between the ages of 26-49. Samples for spot-urine and blood (Day 1-Challenge and Day 2-Fasting) were collected and analyzed. Urinary biomarkers of exposure for the following tobacco-related components were determined: nicotine + nine metabolites (NicEQ-T), NNK, benzene, acrolein, PAHs, 1,3-butadiene, acrylamide, crotonaldehyde, o-toluidine, 2-aminonapthalene, 4-ABP and 3-ABP. Urinary biomarkers of effect (isoprostanes $iPF_{2\alpha}$-III and $iPF_{2\alpha}$-VI) were measured to determine oxidative stress. All urinary biomarkers were normalized to urinary creatinine. Blood exposure biomarkers included COHb (carboxyhemoglobin), nicotine and cotinine. COHb was significantly higher in smokers compared to both moist snuff consumers and non-consumers on Days 1 and 2. Serum nicotine, measured on Day 1, showed smokers>moist snuff

consumers>non-consumers; and on Day 2, moist snuff consumers>smokers>non-consumers. Serum cotinine differed significantly among all cohorts with moist snuff consumers>smokers>non-consumers on both days.

All urinary biomarkers principally derived from tobacco combustion by-products were significantly higher in smokers compared to both moist snuff consumers and non-consumers. NicEQ-T differed significantly among all cohorts (moist snuff>smokers>non-consumers) on both days. NNAL (an NNK biomarker) was significantly higher in moist snuff consumers compared to cigarette smokers and non-consumers on Days 1 and 2. The urinary isoprostanes were significantly higher in smokers compared to moist snuff consumers and non-consumers.

In this study, these data indicate: 1) urinary biomarkers of tobacco combustion by-products are significantly reduced in smokeless tobacco consumers over smokers, 2) urinary NNAL results for smokeless consumers are consistent with values in the literature, and 3) blood exposure biomarkers adequately characterize the three cohorts studied.

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Clinical methodology and results for physiological assessments including flow-mediated dilation, carotid intima-media thickness, ankle brachial index, spirometry, and expired carbon monoxide in exclusive cigarette smokers, exclusive moist snuff consumers and non-consumers of tobacco.

Several physiological assessments have been reported in the literature as measures for detecting, predicting and monitoring cardiovascular disease (CVD). These non-invasive techniques were investigated in three exclusive all male cohorts (cigarette smokers, moist snuff consumers, and non-consumers of tobacco) to evaluate potential differences in CVD status. Flow-mediated dilation (FMD), carotid intima-media thickness (CIMT), and ankle brachial index (ABI) were selected to assess CVD endpoints. Secondarily, spirometry and expired carbon monoxide (ECO) were measured to assess lung function.

The three cohorts were age-stratified into four groups: 26-31; 32-37; 38-43; and 44-49. FMD and ABI were measured on Days 1 and 2; the change between days was calculated. CIMT was measured on Day 2 only. For CIMT, a significant age group main effect was observed, demonstrating a tendency toward higher CIMT with age. For FMD, no significant age or cohort main effects were observed at any time-point. For ABI, the only significant difference was observed on Day 1 between smokers and non-consumers of tobacco, with smokers having the lower ABI mean value.

Day 2 spirometry measures (% predicted FVC and % predicted FEV1) were significantly lower in smokers compared to moist snuff consumers and non-consumers. ECO and derived COHb were significantly higher in smokers compared to snuff consumers and non-consumers. No differences were observed between snuff consumers and non-consumers for either spirometry or ECO.

Although some cohort or age differences were observed in CVD endpoints, the results are consistent with "within normal ranges" for each physiological assessment. ECO measures replicate data reported in other tobacco studies. Spirometry measures support lung function changes expected in smokers compared to smokeless tobacco consumers or non-consumers of tobacco.

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Pyrolysis and combustion of tobacco in a cigarette combustion simulator - Analysis by time-of-flight mass spectrometry with soft ionisation.

The standard methods for analysis of cigarette smoke are mostly offline measurements such as GC-MS. However, tobacco smoke is an extremely complex mixture of particles and vapours, containing thousands of compounds some of which are harmful and can undergo rapid changes in concentration. The application of on-line measurement methods such as time-of-flight mass spectrometry (TOFMS) combined with a soft ionisation technique is expedient to analyse such difficult mixtures. Single photon ionisation (SPI) is a soft ionisation technique with almost no fragmentation. Additionally, the energy of the vacuum ultraviolet (VUV)-photons generated by a laser (E = 10.49 eV) or by an argon (Ar)-excimer lamp (E = 9.8 eV) is insufficient to ionise matrix constituents such as nitrogen. Subsequent TOF-MS parameters allow the detection of organic compounds with a high time resolution. In this regard, a cigarette combustion simulator is used for pyrolysis of tobacco. The simulator is designed to incinerate/pyrolyse a sample in close approximation to the burning conditions experienced by a lit cigarette. It allows parameters such as smouldering and puff temperature as well as combustion rate and puffing volume to be varied and controlled. The objective of the study was to compare smoke chemical composition generated by the simulator and a real cigarette.

The smoking simulator was coupled to the mass spectrometer by a specially designed adapter that enables the analysis of pyrolysis and combustion gases of tobacco and tobacco products (e.g. 3R4F reference cigarette) with almost no aging. The measurements are not influenced by dead volume or memory effects. This facilitates investigation of several toxicants' formation under different puffing conditions (e.g. based on ISO and Canadian intense regimes) and other parameters (e.g. change of puff and smouldering temperature, nitrogen atmosphere). The measurement enables distinction between the different smoking conditions on the basis of the corresponding mass spectra and the results of their statistical evaluations (e.g. Fisher value, principal component analysis). The formation of five selected substances are monitored in more detail: Nitrogen monoxide, Acetaldehyde, 1,3-Butadiene, Benzene and Phenol.

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A fast and simple method for the determination of urinary 1-Hydroxypyrene.

The determination of total 1-Hydroxypyrene (1-OH-Py), a pyrene metabolite, in human urine is used as a biological indicator for exposure to PAHs, which is related to the combustion of organic material. Smoking, living in urban environments and eating grilled or smoked food contribute to the levels detected in humans. In this paper, a fast method for the measurement of metabolites of pyrene in urine was improved by high performance liquid chromatography with fluorescence detector using "heart-cut" technique. This method can quantify the total amount of pyrene metabolites corresponding to glucuronic acid and sulfate conjugates as well as free 1-OH-Py. The hydrolysed biological fluid was directly injected into the chromatographic system, via a column-switching system. Pre-treatment and analysis were performed within 0.5 and 9 min, respectively. Enzymatic hydrolysis has been optimised to not exceed 2 hours. The best response function, in the 0.2-10 ng/mL range, is the linear regression,

bringing simplicity, good accuracy and low quantitation limit. The intra- and inter-day precision values were less than 1 and 2%, respectively. The proposed method provided a simple, convenient and practical procedure to determine the level of the main urinary pyrene metabolites in biological samples. For one hundred samples, the handling time of this on-line method is four times less than off-line methods. Of course, these handling times are directly linked with the cost of the analysis. This method offers also the advantage that the reduction of handling operations decreases the associated risk of error, and, the requested urine sample is low, less than 1 mL, which facilitates biological sampling and transport.

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Limitations in the characterisation of cigarette brands using different machine smoking regimes.

Public health representatives have proposed that the intense regime mandated for testing in Canada with 100% vent blocking, should be used for product characterisation. However the conditions generated in the cigarette during such intense machine smoking do not fit well with most human smoking as shown from this study on ventilated (50%) "3 mg" and unventilated "12 mg" ISO tar yielding cigarettes. Cigarettes were smoked by four machine smoking regimes (ISO, Massachusetts, ISO Working Group 9 Option B and Canadian) and by machine-duplicating human smoking (Sodim DFC D87) from data on 30 smokers of each cigarette type. The puffing conditions of the "average smoker" under laboratory conditions were selected using statistical tools (kernel density computing) and shown to be equivalent to the 90th percentile when the studied smokers smoked under natural conditions (Yield-in-Use protocol by the analysis of spent filters from human-smoked cigarettes).

In this study we show that machine smoking particularly the Canadian regime with the 100% vent blocking does not well reflect how smokers modify their behaviour, on a per puff basis, reducing their smoking intensity in response to increases in draw resistance, smoke concentrations and smoke temperatures. In fact the Canadian regime gives neither smoke temperature nor pressure drop values that were readily achieved by most human smokers.

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A novel indirect inhibitive immunoassay for determination of nicotine in human fluids.

A number of methods utilizing HPLC with UV, electrochemical and fluorescence detectors, gas chromatography-mass spectrometry (GC-MS) and capillary electrophoresis have been used for the determination of nicotine (NIC) in environmental waters and biological fluids. With the development of biosensors, it may be an attractive alternative to existing analytical techniques for the detection of low concentrations of nicotine. The Biacore biosensor, based on the principle of surface plasmon resonance (SPR), is an optical device that detects changes in mass concentration at the sensor chip surface in real time. A new indirect inhibitive immunoassay using surface plasmon resonance (SPR) coupled with molecularly imprinted polymer (MIP) was developed and applied to analysis of NIC. A NIC-MIP coating capillary was produced by *in situ* polymerization technique and used as on-line solid phase extraction (SPE) tube before SPR detection. The anti-NIC mono-antibody was inhibited by NIC that was extracted by the MIP coating in a dose-dependent manner. The calibration curve was generated by linear fit over the range of 0.04-10.00 ng/mL. The detection limit was 0.01 ng/mL, which is lower than traditional immunoassay methods. This method has high sensitivity and can be performed automatically.

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Factors affecting carbon filter adsorption in cigarette smoke.

Cigarette smoke is a complex mixture containing various toxic species which constitute a serious health risk in the vapour phases. Compared to the other adsorbents, porous carbons are considered as the most effective and convenient sorbents for removal of vapour phase toxicants in cigarette smoke owing to its unique physical and chemical properties. However, the effect of tar deposition on the performance of porous carbons is poorly understood. The objective of this study is to investigate the contribution of tar deposition to decreasing carbon activity in a cigarette filter and subsequently what the important carbon variables are to minimize this. Also we aim to establish the relative constructions towards porous carbon deactivation from the smoke particulate phase and the smoke vapour phase and what carbon parameters give the greatest adsorption in the cigarette filter and what types of compounds are adsorbed.

A series of porous carbons with different pore structure and surface chemistry were used as adsorbents. Cambridge filter pads were used to remove the tar in the cigarette smoke. In order to obtain different degrees of tar deposition, the location and number of Cambridge filter pads in a cigarette filter were varied.

Tar deposition has a complex effect on the adsorption ability of the porous carbon. In general, porous carbon protected with a small Cambridge pad reduced the tar deposition on the carbon resulting in a greater adsorption capability for vapour phase smoke toxicants. Using commercial coconut carbon, the deposition of tar on the surface hindered the adsorption of the majority of vapour phase smoke toxicants measured. Further studies using other porous carbons resulted in a similar conclusion.

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Determination of free amino acids with multiphoton-excited fluorescence detection based on nano-scale channel electrophoresis separation.

As a novel nano-technology, nano-scale channel electrophoresis separation (NCS) technique (a hole or channel 0.1-500 nanometer in diameter) is widely applied in biomedical, chemical fields, and so on. Multiphoton-excited fluorescence (MPEF) takes advantages of high spatial resolution (sub-fL level) and high sensitivity (single molecule). Especially, MPEF only occurs in an ultra-low volume (about 100 aL) in the vicinity of the focus, which is perfectly adequate for NCS. In order to provide a new method for an effective separation and detection of biological components, MPEF experiment has been conducted with three fluorescent molecular of different capillary sizes, the results showed that multiphoton-excited volume did not relate to the dimension of capillary. Based on that, NCS was coupled with MPEF detection to analyze amino acids, and 21 amino acids mixture tagged with fluorescein isothiocyanate have been completely separated and sensitively detected. Nine amino acid samples at different concentrations were determined with NCS-MPEF, the calibration curve for each amino acid was established with the linear dynamic ranges above two orders of magnitudes and the linear correlation coefficients $\gamma > 0.99$. It was shown that the system was qualified for the analysis of amino acids. Furthermore, the free amino acids in tobacco leaf were successfully analyzed. NCS-MPEF takes the advantages of aL-level excitation volume and nano-scale channel electrophoresis separation and could be further applied to complex sample analysis.

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The development and application of a method for the estimation of mouth level exposure (MLE) to four tobacco specific nitrosamines.

Tobacco specific nitrosamines (TSNA) in cigarette smoke are an important class of toxicants. Therefore the quantification of TSNA via spent filters from smokers is a valuable addition to the filter analysis method. Previous research has shown that analysis of nicotine in filters gives good correlations to the levels of TSNA measured in smoke.

We have recently described an LC-MS/MS method for the quantitation of four TSNA (NNK, NNN, NAB, NAT); calibration equations with linear correlations were obtained by measuring the mainstream smoke TSNA yields and nicotine levels in filters using six regimes to cover yields that span those expected from human smoked cigarettes. Good correlation was found between TSNA yields and levels of tip nicotine (r^2>0.93) for both a commercial 6 mg (ISO tar yield) product and a 6 mg test product with different tobacco blend and substantially lower mainstream TSNA yields.

In order to demonstrate the methodology, TSNA yields were estimated from filter samples from a previous study where 73 smokers smoked the commercial product from days 1 to 15 and days 51 to 71 and the test product from days 16 to 50. Mean (SD) NNK MLE levels were 51.1 (17.5) and 51.3 (16.7) ng/cig for the control cigarette and 14.8 (6.0) ng/cig for the test cigarette. Other TSNA (NNN, NAB, NAT) were consistent with this trend. Urinary TSNA biomarker data supported these results showing reductions in TSNA excretion (e.g. total NNAL) during the consumption of the test product and returned to the previous levels when the smokers resumed smoking the commercial product. The MLE method is thus able to discriminate the TSNA exposure of smokers following switching to a cigarette with reduced blend TSNA test product.

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Overestimation of tar yields at Canadian intense smoking regime: Factors affecting the accuracy.

The Canadian Intense (CI) smoking regime was derived from the existing ISO smoking procedure but no studies have been conducted on the impact of the intense regime on tar yield measurement. Recently, the ISO TC126 working group WG10 has been created to examine intense smoking regimes. In addition, questions have been raised by the scientific community regarding the accuracy of cigarette tar yields obtained at the CI smoking regime. To better understand the factors impacting tar determination at the CI smoking regime, Total Particulate Matter (TPM) determination was performed using 2 methods: weighing the Cambridge Filter Pad (CFP) and the holder together (ISO method) and separately (modified method), before and after smoking. The modified method allowed determining the exact mass of TPM from which nicotine and water yields are measured. It was shown that the ISO method overestimated by about 12 to 14% the mass of TPM that is actually extracted which resulted in a tar yield overestimation of about 20%. This difference in TPM mass was partly caused by volatile components released from the CFP holder upon its opening and also by residual mass left in the holder. Although several volatile organic compounds have been identified in the headspace of the holder, water was responsible for at least 50% of the TPM mass difference. More accurate values were obtained for TPM and tar by using the modified method. In addition, those tar yields better reflected the maximum exposure level observed in yield-in-use studies conducted with Canadian smokers. The use of the CI tar yields determined with the modified method lead to calculated tar/nicotine ratios similar to those obtained at the ISO smoking regime.

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Baseline data from a longitudinal study to monitor smoking behaviour of smokers of a 10 mg ISO tar cigarette in Germany.

The long-term health effects of cigarette smoking have been extensively investigated and are well known. Many studies have reported the effects of short-term switching on smoking behaviour. However, few long-term studies have been conducted recently involving the monitoring of cigarette consumption, mouth level exposure to nicotine and tar, biomarkers of exposure for nicotine as well as spontaneous switching.

A longitudinal study (clinical trial number ISRCTN95019245) comprising over 1000 smokers of a commercial 10 mg ISO tar cigarette is being conducted to monitor smoking behaviour for up to 5 years, with six-monthly follow-ups. Results from the first time point based on 1019 smokers (on an intention to treat basis) include:

- Cigarette consumption determined by counting smoked filters from cigarettes consumed during a 24 hour period, mean 15.4 cigarettes/day, standard deviation 6.5 cigarettes/day
- Mouth level exposure of nicotine measured by filter tip analysis, mean 23.5 mg/day, standard deviation 12.8 mg/day
- Mouth level exposure of tar measured by filter tip analysis, mean 277 mg/day, standard deviation 150 mg/day
- Nicotine uptake, based on nicotine + 5 metabolites in 24-hour urine, mean 13.9 mg/day, standard deviation 8.9 mg/day
- Nicotine uptake from measurement of salivary cotinine, mean 265 ng/ mL, standard deviation 166 ng/mL

Six-monthly follow-ups will assess smoking behaviour, nicotine uptake, product switching and cessation, and will include reasons for switching and the SF-36v2 Health Survey questionnaire.

Initial data show that the mouth level exposure to nicotine and tar are comparable to data from previous studies. In addition, the correlation of mouth level exposure to nicotine uptake (r=0.51) was consistent with published data. These data support the validity of the parameters being measured.

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Risk assessment paradigms for tobacco smoke constituents.

We propose that product modifications aimed at tobacco harm reduction would be facilitated by identification of those key constituents of tobacco smoke that have toxicological profiles associated with specific diseases caused by cigarette smoking.

Method: One methodology that British American Tobacco (BAT) has adopted uses the margins of exposure (MOE) model, following European Food Safety Authority (EFSA) guidelines. This model permits evaluation of both genotoxic and carcinogenic compounds and in our hands, is applicable to a range of toxicants found in tobacco smoke. An MOE is effectively a ratio between a benchmark dose (derived from existing toxicological dose-response data) and the specific human exposure. Compounds with computed MOEs >10,000 accompanied by logic dialogue are considered to be "low priority for risk management actions". In our calculations, all assumptions regarding cigarette smoke

toxicant yields and retention levels in smokers have been maximised to produce the most conservative MOE values.

Results: Using formaldehyde as an example, four publications covering 10 experimental groups with respiratory tumour endpoints were available to derive MOEs ranging from 102 to 489. These values indicate a high priority for exposure reduction research for formaldehyde within tobacco smoke.

Using an additional mode of action (MOA) approach, we propose that tobacco smoke constituents which are both structurally and toxicologically related can also be grouped to conduct cumulative risk assessment.

Conclusion: Our next challenge is to develop an improved risk assessment paradigm to account for the sequential acute inhalation exposures experienced by smokers. This approach will include the utilisation of contemporary tools such as physiologically based pharmacokinetic (PBPK) models and computational fluid dynamics (CFD) to improve the estimation of human exposure to individual tobacco smoke toxicants during smoking.

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Some statistical considerations regarding the testing of LIP cigarettes.

Supported by Lyapunov's Central Limit Theorem the assumption that measured values are normally distributed is generally made without further consideration. However, for LIP cigarettes this assumption does not hold as is demonstrated in two examples.

First, from theoretical considerations it can be shown that the position of the bands on LIP cigarettes is uniformly distributed. This could also be confirmed by performing a statistical test on the band position measured on 1000 cigarettes.

By evaluating a flow model of an LIP cigarette the influence of the band position on the draw resistance and the degree of filter ventilation are calculated. The results show, that the band position causes variations of about 3% in draw resistance and degree of filter ventilation. The probability distributions of draw resistance and degree of filter ventilation are also calculated by simulation, based on the assumption that the band position is the only source of variation. A comparison with experimental data shows that depending on the cigarette design a statistically significant deviation from a normal distribution can be proved for the open draw resistance but not for the degree of filter ventilation.

As a second example for non-normal probability distributions, arguments are given that the self-extinguishment of non-banded LIP cigarettes represents a Poisson process. As a consequence the residual length of an extinguished LIP cigarette is shown to be exponentially distributed and closed form expressions for mean and standard deviation of the residual length are derived. A comparison of previously published data with the theoretical results on the mean residual length and on its standard deviation for LIP cigarettes with different ASTM pass rates shows exceptionally good quantitative and qualitative agreement. This demonstrates that there is also a theoretical justification for using residual lengths as an additional indicator for the ignition strength of cigarettes.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST19

Bronchial epithelial cell (NCI-H292) response to cigarette smoke total particulate matter (TPM): Development and utilisation of a simple cell culture model of chronic obstructive pulmonary disease (COPD).

COPD is a complex and multifaceted condition that can be caused by cigarette smoking. In smokers, chronic exposure can lead to the development of COPD specific pathologies such as emphysema, small airway disease and mucus hyper-secretion. On a cellular level these pathologies are driven by an abnormal array of exogenously secreted mediators. Here we describe the development and utilisation of a simple cell culture model in which COPD associated mediators are measured following exposure to TPM.

Methods: Confluent monolayers of human lung epithelial cells (NCI-H292) were prepared and exposed for 24 hours to TPM from University of Kentucky 3R4F reference cigarettes. The cytotoxic (EC_{50}: concentration of TPM that kills 50% of the cells) and secretory response (inflammatory, remodelling and mucin mediators) to TPM exposure were then measured using neutral red uptake, electrochemiluminescence detection (MesoScale Discovery; MSD) and enzyme linked immunosorbent assay (ELISA) respectively. Subsequently, similar measurements were undertaken using TPM derived from Reduced Toxicant Prototypes (RTPs) and their commercial controls.

Results: 3R4F TPM induced a concentration dependent cytotoxic response. At non-cytotoxic concentrations (50 and 10 µg/ml) an increase in the levels of COPD associated mediators were also observed. User variability, assay reproducibility and repeatability were all found to be within acceptable limits. No significant difference in the cytotoxic response was seen following exposure to RTP TPMs, their commercial controls or 3R4F. Further analysis demonstrated a significant effect of both dose and cigarette design on mediator release.

Conclusions: This simple cell culture model is able to discriminate between different cigarette designs and is a suitable model in which to assess lung cell response to cigarette smoke TPM.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST20

Comparison of cigarette smoke genotoxicity in three testing systems using mammalian cells.

To aid in the risk assessment of cigarette smoke condensates (CSCs) CORESTA *In Vitro* Toxicology Task Force recommends the following assays for genotoxicity testing in mammalian cells: *in vitro* micronucleus assay (MN), chromosomal aberration assay (CA), and mouse lymphoma assay (MLA). The MN assay is less complex than metaphase analysis in CA and deemed easier than MLA.

The International Conference on Harmonization regards the CA and MLA as equivalent genotoxicity testing; however the MN has not been included in a battery for potential risk assessment. Perceived limitations of the MN assay may be due to an absence of official testing protocol which could result in inconsistent data and as such, test results of the MN have the possibility to differ from those of the CA or MLA.

We reported at the last CORESTA meeting that the conditions of the MN protocol had little influence on the ranking order and ratios of the genotoxic potencies between CSCs.

In this study, three CSCs different in composition were tested in the MN, CA and MLA. Three independent trials in each of the three assays were performed in the presence and absence of exogenous metabolic activation (rat S9).

All assays, irrespective of metabolic activation conditions, gave dose related responses to the different CSCs provided. However, dose-range for responses was assay dependent, with the ranking orders and ratios of genotoxic potencies for condensates in the MN assay being equivalent to those in the CA and MLA.

It is suggested that the MN has the potential to be an alternative assay to the CA and MLA. Our results would further indicate that the MN assay is a quick, efficient and useful tool for the evaluation of CSC genotoxicity.

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Carbon-centered free radicals as intermediates to mainstream smoke carbonyls.

Free radicals in cigarette smoke may contribute to harm associated with cigarette smoking, more specifically, contribute to oxidative stress and subsequent biological activity. A recent high throughput procedure for relative determination of 14 different carbon-centered free radicals (CCFR), both acyl and alkylaminocarbonyl type, was used in conjunction with traditional carbonyl determination to demonstrate the intermediary role of CCFR in mainstream smoke carbonyl generation. This procedure used the radical scavenger 3-cyanoproxyl radical (3-CNP), which was diluted in acetonitrile and spiked onto a 44 mm fiberglass Cambridge filter pad, having the acetonitrile subsequently evaporated. Directly after the 3-CNP coated pad was an impinger containing acidified 2,4-dinitrophenylhydrazine (DNPH) in acetonitrile: water (80:20). Fresh whole smoke from various cigarettes was passed through 3-CNP coated Cambridge filter pads and impingers. Cigarettes evaluated afforded a representative range of standardized 'tar' yields (by the Cambridge filter method), straight grade (Burley, flue-Cured, and Oriental) cigarettes and included Kentucky reference cigarettes 3R4F and 1R5F. Cigarette smoke was exposed to coated filter pads and impingers using the 35 cc puff / 60 sec interval / 2 sec puff duration / 0% vent block (35/60/2/0) smoking regime. Liquid chromatography tandem mass spectrometry (LC-MS/MS) with precursor ion monitoring was used for detecting the large array of radicals, while HPLC with UV detection was used for the determination of carbonyls trapped by DNPH. Two controls, a blank pad and no pad placed in-line between the cigarette and impinger, were used for comparison. The range of CCFR concentration was related to 'tar' delivery and in selected instances, carbonyl delivery was found to be influenced by the presence of the free radical trap by as much as 74%.

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Biomarkers of tobacco smoke exposure measured in UK smokers and never-smokers.

A clinical benchmarking study was carried out in healthy volunteers based in the UK. The primary objective of this study was to determine the concentrations of selected urinary and blood biomarkers of tobacco smoke exposure in regular smokers of four commercially available Virginia-style cigarette products and never-smokers. A secondary objective was to perform a gender comparison of these values. 76 smokers and 20 never-smokers were recruited, with volunteers who smoked being allocated to size equivalent groups matching their usual brand use. Following a screening visit, volunteers entered a clinical residential period during which urine and blood samples were collected. 24-hour urine samples were analysed for creatinine and for biomarkers of the following tobacco smoke components; nicotine (nicotine equivalents), tobacco specific nitrosamines (NNAL, NNN, NAB, NAT), benzene (SPMA), acrolein (HPMA), 1,3-butadiene (MHBMA) and polycyclic aromatic hydrocarbons (1-OH-pyrene). Blood samples were analysed for carboxyhaemoglobin levels. For all

biomarkers, significantly lower concentrations were found in the blood and urine of never-smokers as compared to smokers ($p<0.0001$). Between smoker groups, the majority of mean corrected biomarker levels differed significantly ($p<0.05$ or less), reflecting a generally positive association with increasing product (ISO) tar yields. Nicotine biomarker levels were marginally but consistently higher in female volunteers, whereas 1-OH-pyrene and HPMA biomarker concentrations (corrected for number of cigarettes smoked) were significantly higher in male volunteers. The lowest mean carboxyhaemoglobin levels were observed for the lowest tar products. Overall, these clinical biomarker data indicate that smokers of lower (ISO) yield cigarette products (1-6 mg tar) have reduced exposure to certain tobacco smoke constituents as compared to smokers of higher (ISO) yield (9-10 mg tar) cigarette products.

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Anti-staining cigarette papers.

The formation of stains and spots on cigarette paper is a well known phenomenon in the cigarette industry. Several studies have already been performed to investigate the causes of spot formation on cigarettes. Developments have been conducted aimed at avoiding or at least reducing spot formation.

Our work gives an overview of different kind of spots observed on cigarettes. Potential sources for staining and spotting related to the several different kinds of spots will be discussed.

We have developed methods to simulate spots on cigarettes at the laboratory scale and to measure the extent of spot formation objectively instead of very subjective visual evaluation.

The impact of the chemical composition of cigarette paper on the distinct tendency to develop spots and stains will be studied and validated with examples.

The influence of different coatings on cigarette papers and their effect on possible spot reduction will be shown. The cigarette paper itself and the possible coating must be considered as an option to control spot formation. Adjustments of the chemical composition of the cigarette paper and modification of possible coatings could lead to a reduction of spotting up to 85%.

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Effect on ASTM test results and carbon monoxide deliveries when sodium alginate bands are on the outside of cigarettes.

Fire safety compliant cigarettes which utilize printing technology for cigarette papers typically have the polymeric solutions applied in bands to the wire side of cigarette paper, which is facing the tobacco. The main reason is to keep the bands less visible to the consumer. However, there are some advantages (as well as disadvantages) to having the printed bands on the outside of the cigarette.

During the manufacture of cigarette papers, water is removed from the bottom side, called wire side. This one-sided water removal of water creates an uneven distribution of filler particles, fibers and air spaces through the thickness of the cigarette paper. The opposite side, called top or felt side, has a greater fraction of filler particles, fiber fines and small pores. In a burning cigarette the ash curls toward the wire side and therefore the wire side is the preferred side to have facing the tobacco column and hold-in the coal. When bands are on the outside of the cigarette, they are slightly farther away from the coal than when they are on the inside; and are also somewhat insulated by the cigarette paper.

Hence, the bands are exposed to less heat from the burning coal and their efficacy is improved, which results in higher pass rate in the ASTM test.

This presentation contrasts some of the differences in both band properties and cigarette performance relative to alginate bands being on the wire side vs. top side; including ASTM performance, carbon monoxide deliveries, porosity and diffusivity in the band region and uniformity of bands. Same base papers were printed under the same process conditions on the wire and also top side. The band properties were evaluated. Cigarettes made with these papers were tested in the ASTM test and for deliveries. In addition to higher pass rate in the ASTM test, other benefits observed with alginate bands on the top side of cigarette paper were higher band porosity, less variable bands, and slightly lower CO deliveries.

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Nanocasting strategy to prepare nanostructured porous carbons.

The design and synthesis of porous carbon with desired macroscopic and microcosmic morphology and surface chemistry have been the focus of intensive research, because of their potential applications in the fields of gas storage, catalyst support, microreactor, adsorption and separation. Traditionally, porous carbons were prepared from plant- or coal-based materials via activation processes using CO_2, $ZnCl_2$, H_3PO_4 as active agents. The resultant porous carbon is often of microporous characteristic. In this study, we introduce a new versatile strategy for creating carbons that are more difficult to synthesize by conventional activation. The synthetic procedure contains the following three steps: i) A selected porous solid as mother template with a specific structure is impregnated with a proper carbon precursor; ii) Polymerization and carbonization of the precursor in the pore system; iii) A replica carbon can be obtained after removing the template. This synthesis procedure is named as nanocasting pathway. As an example, a new kind robust porous carbon with high specific area and very large pore volume, CMK-5, has been prepared. The nitrogen sorption data shows the CMK-5 carbon has high specific area of 1770 m^2g^{-1} and the large pore volume of 2.02 cm^3g^{-1}, and the pore size distributions confirm its bimodal pore system with the pore sizes concentrated at 2.7 and 4.9 nm. The transmission electron microscopy (TEM) image demonstrates the tubular structured ordered mesoporosity. Importantly, varying the microstructure of mother templates, the replica products correspondingly exhibit different structures. Significantly, nanocasting pathway opens the door to the design of highly porous carbons with multifunctional properties, which may be promising adsorbent materials for cigarette filter.

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A new application of online sampling with a microprobe inside a burning cigarette coupled with resonance enhanced multi-photon ionization – time-of-flight mass spectrometry.

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The smoke emitted from burning tobacco comprises over 5000 different substances distributed between particles and vapour phase. Due to the high complexity of cigarette smoke it is challenging to find an analytical real time method which can detect organic trace compounds with a wide dynamic range. Photo ionization time of flight mass spectrometry (PI-TOFMS) has proven to be a useful technique to ionize molecules softly and sensitively without fragmentation. With time of flight mass spectrometry a high temporal resolution can be achieved. Thus it is possible to measure puff-resolved

changes in smoke composition during the smoking cycle of a cigarette. Resonance enhanced multi photon ionization (REMPI) using a pulsed UV laser is a powerful approach to analyze polycyclic aromatic hydrocarbons (PAH) with high selectivity and sensitivity. Both approaches have been applied in this study on 2R4F Kentucky research reference cigarettes smoked under ISO and Canadian puffing parameters.

Until now most published puff-resolved and/or on-line studies have been carried out on smoke exiting the cigarette filter. However, for a better fundamental understanding of the combustion/pyrolysis processes during smoke generation, it is desirable to analyse these complex reactions directly inside the burning coal along the tobacco rod. For this purpose a heated microprobe was constructed which samples directly inside the burning coal. Results obtained from this microprobe linked with REMPI-TOFMS will be presented to demonstrate the puff resolved on-line analysis of semi-volatile (poly)aromatic species (e.g. benzene, toluene, phenol, indol, and phenanthrene) inside the burning coal. The gas mixture reveals different behaviour especially in the coal as compared to mainstream smoke yields at the filter. Furthermore this new sampling technique allows sufficient intra-puff resolution and shows significant differences in the temporal course of yield changes during one puff (particularly pronounced for e.g. indol, trimethylbenzene, and phenanthrene).

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT07

A factorial experimental design to investigate the influence of band diffusivity and filler, fibre and citrate contents on the machine smoking yields and LIP performance of banded LIP papers.

Lower Ignition Propensity (LIP) cigarettes are modified cigarettes that demonstrate lowered ignition propensity under specific laboratory tests. These cigarettes must meet standard performance criteria described by the test method established by ASTM E2187-04 regulations in which lit cigarettes must self-extinguish under controlled conditions.

A factorial matrix of eighteen papers was manufactured in order to investigate how the fibre, filler and citrate contents of the paper, together with the band diffusivity and blend density, influence the cigarette LIP performance and machine smoking yields of NFDPM, nicotine and CO. The aim of the project was to determine the factors that influence LIP pass-rates and compare the results with an earlier study that investigated the effects of filler, fibre and permeability on papers with no burn-additive.

A multiple regression analysis of the LIP performance data in Minitab 15 showed that the band diffusivity and the chalk and fibre contents of the paper all significantly influenced LIP pass-rates and residual lengths. Increasing the band diffusivity specification from 0.07 $cm.s^{-1}$ to 0.12 $cm.s^{-1}$ decreased LIP pass-rates and residual lengths by the largest amount. Additionally, increasing the level of chalk in the paper and/or increasing the amount of fibre also had a significant detrimental effect on LIP pass-rates and residual lengths. These data can therefore be used to demonstrate why the cigarettes manufactured from papers with the highest levels of chalk, fibre and citrate burn additive had the lowest pass-rates in LIP testing.

Blend type had the largest significant influence on machine smoking yields of NFDPM, nicotine and CO and puff numbers, with the lower blend density giving lower yields and puff numbers. Increasing the chalk content also significantly decreased yields and puff numbers. Decreasing the fibre content of the paper had a significant effect in reducing CO yields.

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Cigarette peripheral combustion temperature - the effect of smoking regime and cigarette filter ventilation.

Cigarette combustion temperature is an important parameter to understand mainstream and sidestream smoke formation processes. For example, Baker conducted extensive experiments to establish basic thermophysics inside a burning cigarette. However, until recently, most work was conducted with cigarettes of 15 mg ISO tar or above, as available at the time, and often under a single standard machine smoking regime.

Modern cigarettes have numerous design features such as the use of expanded or reconstituted tobacco, and/or filter ventilation. Various intensive smoking regimes have since become increasingly important for regulatory considerations.

In this study, peripheral combustion temperatures from 3R4F Kentucky Research Reference cigarettes were measured using an infrared thermo-camera under a range of filter ventilations (0%, 20%, 36%, 60% and 80%) and three smoking regimes (55 mL/2 s/30 s, 45 mL/2 s/30 s, and 35 mL/2 s/60 s). Maximum peak puff temperatures, average peak puff temperature, and average smouldering temperature were measured systematically. The results indicate that the filter ventilation levels and the smoking regimes have a statistically insignificant effect on the maximum peak puff temperatures. For example, it was found that the maximum peak puff temperature ranged from 1100-1200 °C for both 0% ventilation with 35 mL/2 s/60 s and 80% ventilation with 55 mL/2 s/30 s, at the extremes of the test matrix. The smoke yields for all test products for CO, nicotine, water and NFDPM (Nicotine Free Dry Particulate Matter) were measured and increased with reducing ventilation and increasing intensity of smoking regime.

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Research on relationships between the steric accessibilities of the functional groups of some ambergris-type compounds and their fragrance.

The ambergris is one of the most highly valued perfumery materials, and plays an important role in cigarette perfumery because of its delicate note and good fixative properties. For improving the validity and practicability of relationship between structure and activity of ambergris compounds, the parameters of steric accessibility of oxygen atom and methyl functional group for 28 ambergris compounds were obtained by using quantum chemical calculation method, and the relationships between these parameters and ambergris fragrance were discussed. The results showed that: the ambergris activity of these compounds obviously correlated with the steric accessibility of oxygen atom, which was more than 7 $Å^2$ in all tested active ambergris compounds; once the parameter value was less than 7 $Å^2$, the compounds would become inactive. Furthermore, there was a methyl functional group between the two cyclohexanes in all studied decalin compounds, and its steric accessibility also affected the activity of ambergris compounds obviously. When the steric accessibility of methyl functional group was less than 26 $Å^2$, the compounds did not possess ambergris fragrance either. The results not only enrich the understanding about correlation of molecular structure with ambergris fragrance, but also provide valuable reference for new ambergris molecule designing.

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Determination of chlorophyll metabolites in tobacco leaf by LC/PDA and APCI/MS.

The substantial decrease of chlorophyll in tobacco leaf due to the curing process is commonly known. Although Oriental leaf still gives green colour even after curing, little attention has been paid to the detailed composition of the remaining green pigments. This study aimed to identify the green pigments and quantify them in various tobacco leaves. To this end, liquid chromatograph (LC) concurrently equipped with a photo diode array detector (PDA; 190-800 nm) and atmospheric pressure chemical ionization / mass spectrometer (APCI/MS; 100-1350 m/z) was selected, because it was considered useful for detecting low polar non-volatile compounds giving green colour. Identification was based on the wavelength absorption spectrum, the mass spectrum and the comparison of analytes with synthesized green pigments. Consequently, several chlorophyll metabolites such as hydroxy pheophytin, solanesyl pheophorbide and hydroxy solanesyl pheophorbide etc. were newly identified, other than typical green pigments such as chlorophyll and pheophytin. The quantification was conducted on the following conditions: column; Excelpak SIL-C18 5C^{TM} (250 mm x 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% (B 100%) holds until 55 min, column temperature; 25 °C, detection; PDA at 407, 430, 455 and 660 nm. The composition suggested that chlorophyll metabolites were the main components among green pigments. Additionally, the results showed a larger amount of green pigments in Oriental leaf than other tobacco leaves. Meanwhile, some tobacco leaves such as *Nicotiana rustica* showed different composition, because it does not include solanesol that might relate to the formation of solanesyl pheophorbide or hydroxy solanesyl pheophorbide.

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Nicotine particle / gas phase distribution denuder tube studies.

Mainstream cigarette smoke comprises a complex dynamic mixture of substances with several thousand compounds being generated from the incomplete combustion of tobacco. These substances are distributed in gaseous ('vapour phase') and particle phases of the smoke aerosol. In some instances, a particular substance may occur both in the gas ('vapour phase') and particle phases. Denuder tube technology has the capability of investigating particle / gas phase equilibria, and has been applied to study nicotine particle / gas phase distribution phenomena in mainstream cigarette smoke. The construction of an advanced denuder tube system was undertaken with the objective to improve understanding on nicotine behaviour. This system will be described, with particular emphasis on controlled environmental and/or smoking parameters such as relative humidity (30 - 60% RH), flow rate (5 cm^3 s^{-1}) and denuder temperature (298, 310 K). Results will be presented involving the effect of smoking relative humidity and experimental temperature on the particle / gas phase distribution equilibria of mainstream smoke nicotine and the consistency of the technique. Initial trends indicate that gas phase nicotine is suppressed as smoking RH is increased and as experimental temperature is decreased.

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A clinical trial of cardiovascular disease (CVD) biomarkers in adult smokers and moist snuff consumers. Part I: Study design, subject selection and cohort characterization.

A single site, three cohort, age-stratified, cross-sectional study was conducted in the US in subjects who were exclusive cigarette smokers (n=60), exclusive moist snuff consumers (n=48), and non-consumers of tobacco (n=60) to identify potential CVD-related endpoints that differed among the three cohorts. Enrolled subjects were generally healthy, adult males (ages 26-49) who were free of clinically significant health problems, measured ≥70% of predicted for FEV_1 (spirometry), and were willing to undergo all study procedures. Tobacco-use cohorts provided their usual brand (UB cigarettes or moist snuff) for use during an overnight clinical confinement. On Day 1, a 45-minute product abstention period was followed by a UB tobacco product "challenge" appropriate to the cohort. A 10-12 hour overnight "fast" from food/drink and all tobacco products preceded the start of Day 2 procedures. Clinical endpoints, measured 15-minutes Post-challenge on Day 1 and/or Fasting on Day 2, included tobacco- (and potentially CVD-) related biomarker evaluations of spot-urine and blood samples; physiological assessments (flow-mediated dilation, carotid intima-media thickness, ankle brachial index, spirometry and expired CO); and self-reported product use, nicotine dependence and diet/health status measures. Cohort-specific inclusion/exclusion criteria were well-defined to create exclusive use groups with the expectation that the biomarkers of exposure and effect could differentiate the three cohorts.

In this study, comparison of the three cohorts revealed that, within each tobacco-use cohort, comparable product usage among the different age groups was reported. No significant differences in the Fagerström nicotine dependence scores were observed within or between the tobacco-use cohorts. Overall, the subjects rated themselves as healthier than the population norm on the health questionnaire (SF-36v2™), with no significant age effects observed and only one significant difference between the tobacco cohorts and non-consumers.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST14

Ethyl carbamate levels in US and Swedish smokeless tobacco products.

To date, researchers have reported 28 chemical agents that contribute to the carcinogenic risk in smokeless tobacco products (STP) including ethyl carbamate (urethane). According to the International Agency for Research on Cancer (IARC), ethyl carbamate is classified as Group 2A, a probable carcinogen. Previous studies have shown that the contents of ethyl carbamate in chewing tobacco were 310 to 380 ng/g dry weight basis (DWB). However, approximately 20 years have passed since these measurements were carried out. Research has shown changes in levels of other toxicants in STPs over this time period. Therefore, a more up-to-date survey on the levels of ethyl carbamate was considered necessary to reflect current STPs on the market.

70 major STPs were sampled from Sweden and the US in October 2008. They consisted of 32 Swedish loose and pouched snus products and 38 US products including chewing tobacco, dry snuff, pellets, moist snuff and plug. The STPs were sampled to include products from all major manufacturers within Sweden and the US. Analysis for ethyl carbamate was undertaken by a contract laboratory using an established method. The absence of measurable levels of ethyl carbamate in contemporary chewing tobacco contrasts with historic data. Only moist snuff, loose snus and pouched

snus contained measurable levels of ethyl carbamate. Amongst these products, a significant number were below detection limits (the "as received" wet weight basis (WWB) limit of detection was 20 ng/g). With moist snuff products the range of values measured were <20 to 688 ng/g WWB, loose snus products <20 to 37 ng/g WWB, and portion snus products <20 to 84 ng/g WWB.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. PT04

Improvement of dithiocarbamates residue analysis method by microwave-assisted extraction.

Extraction techniques using microwave-assisted extraction (MAE) and Soxhlet extraction (SE) are evaluated for determination of Dithiocarbamate fungicide (DTCs) residues. The concentration of CS_2 in both extracts are analyzed by gas chromatography using a flame photometric detector (GC-FPD) without any treatment. MAE was compared with SE for determination of DTCs residues in tobacco. Matrix-matched calibration was necessary for reducing matrix effect in MAE, while the calibration in SE was solvent-based. Recoveries on CS_2 fortified in three types of tobaccos (Burley, flue-cured and Oriental) by both MAE and SE coupled to GC-FPD analysis were 70-120% with relative standard deviation values <20%. The comparable results expressed as CS_2 on tobaccos with naturally incurred DTCs were obtained by both MAE and SE. Limit of quantifications (LOQs) were 0.50 mg/kg in both methods. SE contained some complicated and carefully-handed steps, while MAE was a simple and easy method with a single step. Extraction time per one sample in MAE was above half of that in SE. Moreover, SE needed much amount of some harmful solvents; i.e. hydrochloric acid, sodium hydroxyl and sulfuric acid, although usage of hydrochloric acid per one sample could reduce to 28% in MAE, and MAE did not need sodium hydroxyl and sulfuric acid. MAE was considered as a more effective and feasible method than SE. By introducing MAE technique, the determination of DTCs residue in tobacco could be much improved.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT16

Use of two GC-MS scan techniques for the characterization of tobacco fillers used in cigar products.

The proper classification of tobacco products is very important for regulatory and taxation purposes. Cigar products present a special problem because of a wide variation in sizes, blends and additives used in the tobacco filler, and the nature of the wrapper and binder (if used). Proper classification of little cigars (also known as small cigars or cigarillos) can present problems because of their similarity to some cigarette products. However, attempts to use routine chemical and non-routine measures (e.g., LC-MS-MS) to distinguish cigars from cigarettes based on levels of certain analytes have not been fully satisfactory. Consequently, we characterized several varieties of little cigars with two GC-MS scan techniques: 1) the Direct Silylation Scan (*in situ* silylation of tobacco before analysis), which provides identifications and semi-quantitative data, on acids, humectants, sugars, and certain other compounds (Moldoveanu *et al.*, 46th TCRC, Paper #28); and 2) the HFP Scan (*in situ* extraction of tobacco with hexafluoroisopropanol before analysis), which allows the analysis of the semivolatile compounds ranging, from low molecular-weight ketones to neophytadiene and some sterols (Dong *et al.*, 47th TCRC, Paper #16). Both GC-MS techniques were performed on an Agilent 6890 GC coupled with an Agilent 5972 MS. A DB-5MS capillary GC column (25 m x 0.25 µm film thickness and 0.25 mm ID) was used. The data from our analyses allowed us to distinguish between little cigars with blends typical of large cigars from little cigars containing flue-cured tobaccos and little cigars

containing added sugars and/or humectants. Comparisons will also be made with typical cigarette tobaccos.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST17

The development of a quick online equipment measuring the uniformity in the process of cut tobacco flavouring using the technology of virtual instrument.

In order to evaluate the flavouring uniformity in tobacco processing, we designed a quick online real-time measuring method to detect the volatile ethanol concentration of soluble flavour. The equipment consists of the sensor circuit, data acquisition unit and the data processing program. The sensor circuit includes a reference and a measure electrode, one measures the alcohol concentration of the tested position and the other measures the alcohol concentration of the environment background, thus the noise of the environment background can be eliminated. In the data acquisition unit, a high-precision specialized data acquisition module, Advantech's ADAM-4019, is used. It can convert RS-232 signals into RS-485 signals, and the communication between signal and computer are well established. The LabVIEW data processing program has statistics and analytical functions for recorded data. So, this kind of novel equipment can get the real-time voltage signals of alcohol sensor and convert them to the alcohol concentrations value, in digital and wave form.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT28

Influence on filters containing specific additives and synthetic fibres on selected Hoffmann analytes.

Regarding upcoming regulations, non conventional filters can be interesting tools to selectively reduce Hoffmann analytes from the smoke. In this scope, we have studied the influence of filters containing specific additives and different types of synthetic fibres on selected smoke components.

In a first part, we have tested the impact of acid and base additives on paper filters on selected smoke constituents. We have also evaluated the impact of the combination of two filter segments, one acid and the other one based on the smoke yields with a focus on formaldehyde and on the nicotine to tar ratio. For that, paper filters have been impregnated with different types of additives such as tartaric acid, sodium carbonate, polyethyleneimine and sodium glycinate.

Cigarettes have been hand-made with the filters and different types of tobaccos. Depending on the tobacco type, the impact of the filter additive is different.

In a second part, we have tested synthetic fibres and their influence on selected Hoffmann analytes mainly from the volatile and semi-volatile phases of the smoke.

Results will be presented and discussed by deliveries per cigarette and per mg of tar.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT40

An *ex vivo* approach to the cigarette smoke-induced parenchymal responses.

Current experimental approaches to biological effects of cigarette smoke apply either to cell culture or animal models. The former remains weak in providing information concerning cell-cell and cell-matrix interactions. The latter, is used mostly for pathological and rarely for short-term toxicological assessment. Lung slices maintain three dimensional structures of airways and their use minimize the number of animals required to perform experiments. We investigated the feasibility of applying rat lung slice culture for acute cigarette smoke toxicology studies.

Rat lung slices were maintained at the air-liquid interface in Transwell® inserts before being transferred to specially designed chambers and exposed to whole smoke (WS) or vapor phase (VP). They were subjected to a 3-day exposure with WS/VP (3R4F, 30 minutes/day, 70 ml/puff, at a dilution in air of 2%, 5%, 10% (v/v) and sham air control delivered by a Borgwaldt RM20S® Smoking Machine).

Following WS and VP exposure, dose-response related toxicological effects were observed while histological observations were in agreement with the results of the toxicity assay. No differential toxicity was observed between WS and VP exposure. However, when the model was exposed to a 5% diluted WS in air (v/v) from 1, 8 and 14 mg ISO tar yield cigarettes, various levels of toxicity were observed. Cytochrome P450 1A1 gene was significantly induced by either 2% diluted WS or VP in air (v/v). WS appeared to be more potent than VP in such induction.

Thus, we conclude that this lung slice model is suitable for the study of parenchymal responses including toxicity and stress gene induction following acute cigarette smoke exposure.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT23

A novel approach to the study of the dynamic smoke flow between the tipping paper and the filter of ventilated cigarettes.

An understanding of the behaviour of smoke stream between the tipping paper and the filter plug wrapper or between the plug wrap paper and the filter material respectively is essential to adjust the design of a cigarette mouthpiece for a proper control of the expected smoke deliveries. The idea of this study is to investigate the smoke flow pattern underneath the tipping paper for various types and configurations of tipping perforation by using a numerical simulation technique. From Newtonian dynamic relations of viscous fluids and by adapting the Navier-Stokes equation, the aerosol deposition as a result of the mass transfer from smoke stream to the inner tipping paper surface can be simulated. The proposed theoretical approach makes exclusively use of physical and geometrical parameters of individual perforation methods applied to different commercial cigarette brands and provides a simplified image of the laminar flow around perforation holes. The calculated results from the two-dimensional pattern model are compared with the experimental observations which are based on optical and digital analysis of the condensate deposition intensity. The good correlation between the mathematically derived and measured values opens the potential to predict the expected smoke flow pattern solely from the tipping paper permeability adjustment. Furthermore, the observed mass transfer depends on a strong interaction between the tipping paper and filter plug which may be considered by filter manufacturers to control the filtration efficiency of their products.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. PT09

Real-time mainstream gas-phase smoke detection from cigarettes with activated carbon in cavity filter.

A real-time sampling system has been set up to monitor a group of volatile smoke analytes (nitric oxide, acetaldehyde, acetone, benzene, toluene, 1,3 butadiene, isoprene and carbon dioxide) in mainstream cigarette smoke on a puff-resolved basis. The focus was to understand the interaction between activated carbon in filter cavity and the volatile species. Smoke was sampled at the mouth end of the cigarette filter in order to minimise any dead volume. This novel system was able to record a number of detailed gas evolution profiles simultaneously during puffing and interpuff periods without cleaning procedures. The results revealed that not all the smoke generated during the inter-puff smouldering period was released as sidestream smoke. Some smoulder-generated volatile species were trapped by the tobacco rod and were able to contribute to the subsequent mainstream smoke upon puffing. In addition, it was observed that the presence of granulated activated carbon in the filter removed part of this trapped smoke during smouldering, although the majority of the volatile adsorption occurred when the smoke passed through the filter during puffing. The technique is useful in gaining real-time mechanistic insights into the formation of gaseous smoke constituents and their subsequent adsorption by activated carbon.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. PT02

Study on surface free energy and its components of different types of tobacco leaves.

In tobacco manufacture, wetting, which is an important behaviour at the solid-liquid interface, is a common physicochemical phenomenon present in between essences, casing sauce, humectants or water and tobacco surface. Surface free energy (SFE) and its polar and disperse fractions (non-polar fraction) are fundamental properties of solid surfaces, which is relative to anisotropy, roughness, wettability, adhesion and adsorption effects of the solid surface. To study the wettability and spreadability of tobacco leaves, SFE of three tobacco types (Burley, flue-cured, and Oriental) was obtained by measuring the contact angles between probe liquids with different surface tension and tobacco leaves. The aim of the study is to provide the basic data for tobacco technology, especially flavoring and casing. The results indicate that the SFE of three types is close, that is, SFE of Burley tobacco leaves is 25.21 mN/m, SFE of flue-cured tobacco leaves is 25.78 mN/m, and SFE of Oriental tobacco leaves is 27.15 mN/m. However, there is a distinct difference in the proportion of polar and disperse fractions. The disperse fraction of Oriental tobacco leaves is as high as 92.34%, while it is only 41.06% in flue-cured tobacco leaves and 65.53% in Burley tobacco leaves.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SS01

Biomarkers for the assessment of reduced toxicant prototypes (RTPs).

In 2001, the US Institute of Medicine coined the term "Potential Reduced-Exposure Product", referring to novel tobacco products which yield reduced tobacco smoke constituents with a reasonable expectation to lower consumer health risks. Although informative, measurement of chemical smoke constituents alone is not adequate to assess novel products in terms of exposure and harm reduction. Further tools are needed to understand how consumers use novel products, how much of the chemical yield is absorbed by the body, and how to subsequently assess the biological effects and health risks of product use.

Biomarkers are being developed at British American Tobacco to fill the gaps between smoke chemistry and epidemiology for RTP assessment. Biomarkers of exposure can be utilised to measure specific smoke constituents absorbed by the body. Biomarkers of biological effect can yield valuable information regarding the body's response to smoke constituent challenge and pathological processes

potentially leading to disease development. For the purposes of RTP assessment, biomarkers of biological effect would need to demonstrate a robust measure of response to smoking that is minimally affected by inter-individual variability. Furthermore, such biomarkers would need to show a favourable change in the magnitude of response upon smoking cessation. Finally, the amount of time required to see such changes should be suitable for pre-market testing.

Currently, all biomarkers are lacking formal validation, although there is increasing confidence within the scientific community in some biomarkers of exposure. Short-term clinical studies combined with longer term epidemiological studies are needed to understand biomarker behaviour when consumers switch from a conventional product to a RTP. More importantly, the biological relevance of any changes needs to be understood in both individual consumers and the general population before biomarkers can be fully utilised for product assessment.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. PT07

Development of deep-cavity calixarene materials for selectively reducing benzo[a]pyrene and major phenolic compounds in mainstream cigarette smoke.

In order to selectively reduce the contents of benzo[a]pyrene and major phenolic compounds in mainstream cigarette smoke (MCS), two kinds of deep-cavity calixarene powder materials, p-[1-(4-methoxyphenyl)-1-methyl-ethyl] calyx[8]arene (I) and p-[1-(4-hydroxyphenyl)-1-methyl-ethyl] calyx[8]arene (II), were synthesized from p-[1-(4-methoxyphenyl)-1-methyl-ethyl] phenol and characterized by ^{1}H NMR, ^{13}C NMR, fast atom bombardment MS, thermal analysis and element analysis. Cigarette samples were prepared by respectively adding calixarene materials I and II into filter tip at the rate of 10 mg/cig., the results from cigarette smoke analysis showed that the additives had little influence on the deliveries of tar, CO, nicotine and moisture content in MCS. The benzo[a]pyrene content in MCS decreased by 13% and 52% with the addition of I and II, respectively. For additive I, the decrease percentage of hydroquinone, resorcinol and catechol in MCS was all less than 10%, and that of phenol, *o*-cresol, and *m-/p*-cresol in MCS was about 18%. As for additive II, the decrease percentage of hydroquinone, resorcinol and catechol in MCS was 50%-56% and that of phenol, *o*-cresol, and *m-/p*-cresol in MCS was 62%-66%. These experimental results indicated that the synthesized deep-cavity calixarene materials could be used to selectively remove benzo[a]pyrene and major phenolic compounds in MCS.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST09

Correlation between manual and semi automatic measurements of ignition propensity to ASTME2187-04.

The current ASTME2187-04 method for ignition propensity is both time consuming and subject to variability due to the human element of the test. In order to reduce this inherent variability a semi automatic method was developed that relies upon a camera with pattern recognition technology.

The use of such camera technology highlights the difficulty of deciding the length of a burn and several examples are presented where the burn length could be in dispute. Consequently a "rules based" decision making process was adopted to determine a burn as being full length, terminated or questionable.

The apparatus was tested by comparing the full length burn (FLB) of commercially available cigarettes tested under semi automatic conditions with the same brands tested using a manual tool. Good agreement was achieved for the brands tested.

Estimation of the repeatability of the apparatus was also made using FLB and residual length (RL) statistics on commercial cigarettes.

The repeatability was found to be brand dependant. The significance of residual burn length and ASTME2187-04 pass/fail criteria are noted and discussed.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT30

Determination of 21 polycyclic aromatic hydrocarbons in smokeless tobacco products: Method validation, application and comparisons.

An improved method to measure levels of polycyclic aromatic hydrocarbons (PAH) in smokeless tobacco products has been developed and validated. This method utilized base saponification and partitioning steps associated with the analysis of benzo[a]pyrene in whole tobacco (Health Canada Test Method T-307). To extend the number of PAHs determined, eight deuterated PAHs were added as internal standards (IS), as well as a solid phase extraction clean-up through two adsorbents (stacked NH_2 / silica). Analysis was performed by GC/MS in selected-ion-monitoring mode. Recoveries of target PAHs from spiked smokeless tobacco ranged from 84 to 104% with relative standard deviations under 11%. This method was applied to four CORESTA reference products (CR1, CR2, CR3, CR4), a Kentucky reference product (2S3), as well as eight commercially available smokeless products. The target PAHs found in the reference products ranged from 0.82 ng/g for acenaphthene (CR4) to 6754 ng/g for phenanthrene (CR2) on a "dry weight basis". The benzo[a]pyrene results ranged from 1.71 to 100 ng/g and were consistent with values generated by T-307 using HPLC with fluorescence detection. This method differs from that described in a recent publication using direct extraction into cyclohexane and ^{13}C labeled PAH as IS. The published data showed reasonable agreement with those generated using this new method for nearly all PAHs with the exception of naphthalene where the reported values were nearly thirty times higher (1980 ng/g and 68.7 ng/g respectively). Attempts were made to reproduce the published method and in our hands, the two methods yielded comparable results for naphthalene. However, for other PAHs, the extraction efficiencies were two to five times lower than those determined using the new method. Possible explanations for the discrepancy in the results for naphthalene were investigated and the results will be presented.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. PT08

The effect of position of carbon granules within a cigarette filter on vapour phase retention.

Numerous papers have been presented at previous conferences examining the characteristics of filter cigarettes containing activated carbons on the removal of vapour phase compounds from smoke. All these studies have been carried out on triple granular or active acetate ("dalmation") type filters in which the carbon granules are evenly spread across the body of the filter. Innovations in filter manufacture now enable the carbon to be localized in particular cross-sectional regions of the filter, notably around the periphery (as in "Active Patch" filters) or more recently within a centrally located pocket (as in "Smooth Core" filters). This leads to the question of the extent to which the vapour phase retention of such carbon filters is affected by the localized position of the carbon granules. The vapour phase retention will not only be affected by the smoke flow paths relative to the position of the granules, but also by diffusional mechanisms occurring as the smoke travels along the filter. This

paper examines the interaction between the position of carbon granules and the overall vapour phase removal efficiency of the filter.

Experimental findings are presented of the relative retentions of vapour phase compounds, notably carbonyls and hydrocarbons, and semi-volatile compounds, notably phenol and cresols, by four different types of carbon filters - 'Active Acetate', 'Triple Granular', 'Active Patch' and 'Smooth Core'. The results demonstrate that the weight, rather than the position, of the carbon within the filter is the dominant factor affecting retention.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SS07

Measuring the regional deposition of tobacco smoke particles in the human respiratory system.

A study was conducted to determine the regional deposition of tobacco smoke aerosol in the respiratory system of smokers, using real-time measurement of respiration and inhaled and exhaled smoke.

Six current smokers were recruited, giving informed consent. The volunteers smoked a commercial 7 mg ISO tar yield King Size cigarette. Each volunteer smoked 1 cigarette, at each of the following self-perceived inhalation regimes: mouth hold, shallow, normal and deep inhalation. Puffing behaviour was measured by a smoking analyser and respiratory behaviour was measured using the Lifeshirt® system

Exhaled smoke was captured by a sampling system operating at a constant flow of 217 $l.min^{-1}$. A sub-sample was analysed by a real-time particulate spectrometer, which measures particle size and concentration. Inhaled smoke was characterised by replaying the recorded human puffing profiles.

Inhaled and exhaled particulate per puff were calculated as total number and total volume of particles. Exhaled fraction was calculated as the ratio of exhaled to inhaled particulate. Respiratory behaviour was expressed as inhalation depth (percentage of vital capacity), mouth hold, inhalation, exhalation and total residence times in the respiratory system.

Linear regression showed that number and volume weighted exhaled particle fractions were poorly correlated to inhalation depth.

A model was developed for particle deposition driven by residence time, assuming diffusional deposition. The deposition rate was allowed to vary by anatomical region of the respiratory system.

There was a reasonable correlation between predicted and measured exhaled fraction, allowing regional deposition to be estimated. There were significant differences in the regional deposition of smoke with different inhalation regimes, with mouth hold and shallow inhalation resulting in higher deposition in the upper airways, and medium and deep inhalation resulting in higher deposition in the lower airways.

Future work will improve the measurement of respiratory behaviour, particularly the time resolution.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT19

Determination of total alkaloids as nicotine in tobacco by continuous flow analyzer using stable and safer reagents.

ISO 15152:2003 describes analysis of total alkaloids as nicotine (TNA) in tobacco using continuous flow analyzer. 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, a known toxic chemical with LD_{50} of 5 ppm.

In this method, TNA is determined by reaction of an aqueous extract of tobacco with sulfanilic acid and cyanogen chloride. The developed colour is measured at 460 nm. Cyanogen chloride is generated *in situ* by reaction between a potassium thiocyanate and a solid stabilized chlorine donor reagent. 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 optimized to obtain results equivalent to the ISO method.

The method has been validated as per standard validation protocols i.e. limit of detection, limit of quantification, recovery, precision, accuracy and reproducibility. Recoveries of 97% were obtained with linear regression coefficient of 0.9999.

The "r" and 'R" studies on the samples have been conducted with nicotine concentration range from 0.5 to 3.3%. Various tobacco grades with different nicotine concentrations were analyzed simultaneously using both methods and maximum difference in the results were ±0.09 units.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST15

Quantitative determination of reconstituted tobacco in a blend.

Currently, there is no scientific method for the quantitative determination of reconstituted tobacco (recon) in a blend and the most preferred method is the hand picking method, which is very tedious, time consuming and fine recon particles may be ignored.

A simple method is developed for the determination of recon content in a given blend, which is simple, fast and accurate. The method involves distribution of 5 gm of blend in 100 ml of a halogenated solvent in a specially designed separating funnel. The content of flask are thoroughly mixed. Recon settles down based on density from the rest portion of the blend. Recon is separated, dried and calculated as percentage in the blend.

The method is applicable to recon of extruded / paper type involving binders like guar gum and carboxymethyl cellulose etc. Recoveries of 95% are obtained. The method has been applied to various blends containing recon and recoveries are better than the hand-pick method, indicating its superiority over conventional hand picking method.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST21

***In vitro* characterization of eugenol and methyleugenol hepatic bioactivation versus detoxification pathways: A comparative study in rodents and humans.**

Introduction: Cloves are a component of kretek cigarettes and contain significant concentrations of eugenol (CAS-97-53-0). US-NTP (national toxicology program) studies found no evidence for eugenol-induced carcinogenicity in rats, but equivocal evidence for hepatic tumour formation in

female mice. By contrast, the structurally-related compound methyleugenol (CAS-93-15-2) is consistently carcinogenic.

Objective: As part of a risk assessment for the use of eugenol in kreteks, we studied the formation of the 1'hydroxy proximate carcinogen of eugenol and methyleugenol in human and rodent *in vitro* systems.

Method: Pooled human and rodent liver fractions (microsomes, S9) were incubated with ^{14}C-eugenol and ^{14}C-methyleugenol (20 µM final, 1.8 µCi) in the presence of metabolic co-factors. The 1'hydroxy metabolite was quantified by radio-HPLC.

Results: CYP450-dependent formation of the 1'hydroxy genotoxic precursor accounted for 16% and 4% of total eugenol in human liver microsomes and S9 fractions respectively. By contrast, the equivalent 1'hydroxy genotoxic precursor accounted for 22% and 30% of total methyleugenol in human microsomes and S9 fractions respectively. On addition of glucuronide, 85% of eugenol was conjugated and no 1'hydroxy genotoxic precursor was detected. Methyleugenol was not conjugated under identical experimental conditions. Formation of the 1'hydroxy metabolite of eugenol and methyleugenol was similar in human and rat but higher in mouse. Glucuronidation of eugenol was partial in rat and comparable to human in mouse.

Conclusion: Methyleugenol metabolism in the liver leads to accumulation of a 1'hydroxy genotoxic precursor, whereas eugenol bioactivation is limited and glucuronidation is predominant. Increased toxicity potential is observed in rodents due to either higher rate of 1'hydroxy metabolite formation or decreased rate of glucuronidation.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST04

Colorimetric detection of triacetin levels in cigarette filters.

Triacetin is widely used as a plasticizer and one of the most important materials in cellulose acetate filter production. It is essential to check quantity of triacetin in filters because uncontrolled concentrations cause formation of melt holes or too soft filters that subsequently would lead to hot collapse effect while smoking. Traditionally triacetin levels in cigarette filter production are determined by weight comparison method but it is not very accurate, can be used only on the working production line and has other drawbacks. Our proposed method lets us determine triacetin levels in non-cured as well as fully cured filters and align the results with weight comparison method. Fast and accurate colorimetric method of triacetin determination is based on esters conversion to hydroxamic acid which forms purple-red complexes with ferric ion. This reaction is widely used in various esters determination long time. Method was specialized for triacetin detection in cigarette filters with concentrations as low as 3×10^{-4}M. The experimental data showed linear relationship of calibration curve from solution concentrations in the range from 3×10^{-4}M to 10^{-3}M. We believe this practical method has the key advantages of allowing accurate determination of triacetin levels during and after the production of filters without a need for expensive equipment.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST16

Study of technological and chemical characteristics of processed tobacco in various bulk-curings in Guilan.

To study the effect of different bulk curings for tobacco drying on chemical and technological properties of tobacco Coker 347 in Guilan, a plan was implemented in the design of factorial randomized complete block with 3 replications. The project was evaluated as a factor in bulk curing type A in 4 levels and harvest or leaf position as a factor B in 3 levels. Qualitative and quantitative properties of flue-cured tobacco are influenced by many environmental factors, agricultural and processing technology and these factors create a lot of variation in physical and chemical properties and tobacco taste. Meanwhile, technological processing and drying is very important. The different structures of the traditional bulk curing, semi-modern and modern systems that can control temperature, humidity and ventilation, cause tobacco processing differences together in terms of physical, chemical and taste. Traits include percent sugar recovery, percentage of total sugar, percent nicotine, sugar recovery ratio of total sugar, filling power and economic comparison of different bulk curing. Data were analysed with statistical software SAS. Tables of results of analysis of variance show that there is no significant difference between types of bulk curing (factor A) during 2 years in terms of impact on the percentage reducing sugar, total sugar and nicotine content of tobacco, but significant difference at 5% level in terms of impact on the reducing ratio of sugar to total sugar and filling power. Tobacco processing showed another significant difference at 5% level in terms of harvest (factor B) in reducing sugar, total sugar, nicotine, and filling power, but no significant difference in the reducing ratio of sugar to total sugar. Mean treatments using LSD (Least-Squares Deconvolution) for average reducing sugar showed that tobacco cutter-leaf dried in bulk curing corrective JTI (Japan Tobacco International) had the highest average, tobacco cutter-leaf dried in a traditional bulk curing with the highest average total sugar, tips dried leaves in modern bulk curing JTI highest average nicotine, priming-lugs dried in the bulk curing domestic reforms were the highest filling power.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT36

Menthol smokers have lower mouth level exposure to "tar" and nicotine than non-menthol smokers.

Mentholated cigarettes have been subjected to increasing scrutiny. Some critics have suggested that menthol cigarettes are smoked differently from non-menthol cigarettes, resulting in the potential for higher smoke exposure, while recently published biomarker data showed no effect of menthol on exposure. To better understand the effect of menthol on mouth level exposure (MLE) to "tar" and nicotine, a large consumer-based study was carried out in the US.

The study included data from smokers using menthol (M) and non-menthol, (NM) full-flavor (2 M, 3 NM) and lights (2 M, 3 NM), king-size brand styles. Data were obtained from 55-75 smokers of each brand style. In addition to the number of cigarettes smoked during a day, yields of "tar" and nicotine were determined on both a per-cigarette and per-day basis. The data were examined using a two-way ANOVA with "tar" category and menthol as main factors to determine the significance of menthol's effect. The data were also examined using ANOVA within each "tar" category.

The test of overall impact of menthol showed that menthol cigarettes produced lower MLE to "tar" and nicotine on a per-cigarette and per-day basis than non-menthol cigarettes. There was no statistically significant difference in the number of cigarettes smoked. When the effect of menthol was examined by "tar" band, the menthol lights cigarettes resulted in significantly lower MLE exposure to "tar" and nicotine on a per-cigarette and per-day basis. The per-day reduction in MLE was accentuated by menthol lights smokers consuming fewer (2.1, $p = 0.02$) cigarettes per day than the non-menthol smokers. However, no statistically significant differences were observed between the yields or cigarettes per day consumed by full flavor menthol and non-menthol smokers.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT12

Effects of paper and filter on Hoffmann analytes.

To investigate the effects of paper and filter on the harmful components in mainstream cigarette smoke, cigarette paper, tipping paper and plug wrapper with 5 different air permeabilities respectively, cigarette paper with 5 different grammages, acetate filter rod with 5 different draw resistances, were used to manufacture 40 cigarette samples with flue-cured cut tobacco of the same batch. Nine routinely measured components and Hoffmann analytes, including tar, nicotine, CO, HCN, NNK, NH_3, B[a]P, Phenol and crotonaldehyde, in the mainstream smoke of the samples were analyzed and the mathematical regression equations describing the relationship between these analytes and the parameters of cigarette auxiliary materials were obtained. The results showed that: 1) the grammage of cigarette paper affected the deliveries of phenol and CO significantly, the delivery of phenol decreased, while that of CO increased with the rise of grammage of cigarette paper; 2) the air permeability of cigarette paper significantly negatively correlated with seven analytes, which in the order of decreasing extent were HCN>NH_3>B[a]P≈CO>phenol>nicotine>tar; 3) the draw resistance of the filter rod featured an obviously negative correlation with 8 analytes, which in the order of decreasing extent were phenol>NH_3>NNK>tar>HCN>nicotine>B[a]P>CO; 4) the air permeabilities of both tipping paper and plug wrapper affected the deliveries of these nine components, however the influence of tipping paper was stronger than plug wrap, while the influence of plug wrapper increased with the raise of air permeability of tipping paper; 5) the ventilation of filter significantly negatively correlated with eight analytes, which in the order of decreasing extent were HCN>CO>crotonaldehyde>NH_3≈tar>phenol>nicotine>B[a]P.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT41

The role of cigarette smoke constituents in oxidative modification by aqueous extracts of mainstream cigarette smoke. Part I: 8-OHdG.

Oxidative stress is a state of imbalance between reactive oxygen species (ROS), and the ability of the organism to defend against them, leading to progressive oxidative damage. ROS causes oxidation of proteins, DNA and lipids, which may cause direct tissue injury or induce a variety of cellular responses. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the markers for oxidative stress. In this study, we investigated cigarette smoke constituent(s) associated with 8-OHdG formation in human alveolar type 2 epithelium-like adherent cell line A549 induced by whole smoke-bubbled phosphate buffered saline (WS-PBS).

Gas vapour phase-bubbled PBS (GVP-PBS) was estimated to have almost the same level as WS-PBS on 8-OHdG induction in the experiment with a reference cigarette, 3R4F. Correlative analyses between the 8-OHdG induction by 9 different sample cigarettes and the amount of each smoke constituent of them showed that each of the carbonyl compounds (formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, methyl ethyl ketone, n-butylaldehyde) had a high correlation with 8-OHdG induction. When A549 cells were exposed to each of the carbonyl compounds in the amount equivalent to that in WS-PBS of 3R4F, acrolein was the only inducer of 8-OHdG. And the index value of 8-OHdG induction (calculated as the ratio of induction by acrolein to induction by WS-PBS of 3R4F) was 1.00±0.10. Furthermore, when A549 cells were exposed to WS-PBS with acrolein in the amount equivalent to that in WS-PBS of 3R4F, similarly, 8-OHdG was induced, and the index value of 8-OHdG induction was 2.00±0.50. These results show that acrolein is a major factor of intracellular 8-OHdG induction in our study, and they also suggest that acrolein induces 8-OHdG without almost any influence from the other smoke constituents.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SS04

Cardiovascular disease biomarkers study. Part III: Tobacco-related biomarkers of effect in exclusive cigarette smokers, exclusive moist snuff consumers, and non-consumers of tobacco.

A three cohort, age-stratified cross-sectional study was conducted in the U.S. in cigarette smokers (n=60), moist snuff consumers (n=48) and non-consumers of tobacco (n=60) to evaluate several known cardiovascular biomarkers of effect. Subject enrolment was restricted to generally healthy males (26-49 years) who were free of clinically significant health problems, not taking medication for chronic medical disorders, willing to undergo all study procedures including one overnight confinement, tested negative for drugs and alcohol, and measured ≥70% of predicted for FEV_1 on spirometry. Physiological assessments included measures of flow mediated dilation (FMD), ankle-brachial index (ABI) and expired CO (ECO) on Days 1 and 2; carotid intima-media thickness (CIMT) and spirometry on Day 2. Forty-five blood biomarkers of tobacco exposure/effect and fourteen clinical hematology indices were also evaluated. For the cardiovascular-related physiological assessments, no significant differences were found between cohorts for FMD or CIMT although a significant age group main effect was observed for CIMT. Smokers had a lower mean ABI value following normal smoking behavior, but this difference was lost after a 10-12 hour overnight tobacco-abstinence period. ECO was significantly different between the cohort that smoked and the two that did not. Approximately half (n=22) of the measured blood biomarkers of effect showed differences in cohort comparisons. IL-12(p70), ICAM1, IL8 and MCP1 were the biomarkers that best differentiated the three cohorts. Age effects were also seen. In conclusion, significant cohort and age effect differences were identified. Concentrations of measured biomarkers were consistent with normal clinical ranges; however, differences between cohorts (within normal ranges) were observed.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST22

Gene expression analyses of the liver From ApoE-/- mice exposed to mainstream cigarette smoke.

The goal of this study was to identify key molecular alterations induced by mainstream smoke exposure and diet in a genetically susceptible mouse model of atherosclerosis. ApoE-deficient female mice were exposed nose-only to mainstream cigarette smoke for 18 weeks (3 h/day, 5 day/week) at concentrations of 0, 0.16, 0.32 and 0.48 mg WTPM/L +/- high-fat diet. Total RNA from the liver was isolated and prepared for hybridization onto Affymetrix 430 mouse 2.0 arrays. Using a Benjamini and Hochberg False Discovery Rate for multiple testing correction ($p<0.05$) and a 2-fold threshold, ANOVA analysis identified 1233 differentially expressed genes compared to control samples. Genes having the greatest change from basal expression included: SQLE, GPNMB, IDI1, CYP51, LEPR, SC4MOL, SULT1E1, FAM19A2, CYP2C55, and FDPS. The top gene ontologies included lipid and immune/inflammatory processes. Gene expression in the liver from mice fed a high-fat diet was significantly modulated compared to chow-fed controls. Livers from mice exposed to cigarette smoke and consuming the high-fat diet had the highest number of differentially expressed genes compared to controls, whereas the chow-fed mice exposed to smoke had the fewest. In summary, the combination

of diet and mainstream cigarette smoke exposure had the biggest effect on molecular alterations in the livers of ApoE-/- mice.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST23

The development of an anchorage independent growth assay for the screening of tobacco products.

Cancer is a multi-stage process which can be broken down into 3 separate phases: initiation, promotion and progression. Malignant transformation occurs in the progression phase. Hallmarks include uncontrolled proliferation and the subsequent loss of growth inhibition. These phenotypic changes can be assessed using the anchorage-independent growth (AIG) assay where cells are transferred to a semi-solid medium and assessed for their ability to grow.

Here we describe the development of the AIG assay for the assessment of tobacco products. Specifically, anchorage-dependent BEAS-2B cells, a human bronchial epithelial cell line, were exposed to total particulate matter (TPM) for 72 hours. Following exposure, cells were plated in Noble agar in 6- or 12-well plates. In parallel, cytotoxicity was assessed using the CellTiter-Glo® assay. Anchorage-independent A549 cells were used as a positive control to ensure the assay conditions were suitable for colony formation and growth. The cells plated in agar were incubated at 37 °C, and colonies were scored after 21 days using a GelCount™ colony counter.

Results demonstrate that BEAS-2B cells exposed to TPM form colonies in agar. The number of colonies was not dose-dependent and was not consistent across experiments. Statistical analysis showed that the variability in response was not due to passage number, choice of culture medium, or soft agar components. This suggests that cigarette smoke TPM failed to drive AIG under the conditions tested.

In order to determine whether the vapour phase contributes to AIG in BEAS-2B cells, this model is being further developed with whole smoke as the exposure agent. The successful outcome of this follow-up study could confirm our assumption that the BEAS-2B cell AIG assay is a useful *in vitro* model for investigating the carcinogenic potential of tobacco products.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT11

Alternative analytical methods for the determination of selected volatile Hoffmann analytes in mainstream cigarette smoke by GC-MS: Method development and validation.

The necessity to analyse volatile organic compounds from the Hoffmann list has led to numerous analytical procedures for their quantification. Methods for the determination of these substances were also published by Health Canada, but only selected validation parameters have been reported so far.

Recently, a collaborative study for the following volatile organic compounds: isoprene, toluene, benzene, butadiene, acrylonitrile was carried out by the CORESTA Special Analytes Task Force and a CORESTA Recommended Method will be available in 2010.

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 - such as isoprene, toluene, benzene, butadiene, acrylonitrile - and additionally various carbonyls, e.g. acetone, acrolein and acetaldehyde, as well as hydrogen cyanide in cigarette smoke was developed and validated following

international guidelines. For this purpose the gas phase of cigarette smoke is sampled in a Tedlar bag and an aliquot of the gas phase is subsequently analysed by GC-MS. Calculation of results is carried out using deuterated internal standards, e.g. d_6-acetone, d_6-benzene and d_8-toluene.

The cigarette samples selected for this study covered a range of ultra low tar products (ISO smoking regime) up to 36 mg NFDPM per cigarette (Canadian Intense smoking regime). 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, recovery, and the possibility to compare the results obtained by this method and other alternative methodologies will be discussed.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST24

Standardization of the preparation of smokeless tobacco extracts for assessment of biological effects.

Exposure to smokeless tobacco (ST) has been reported to elicit diverse biological effects at the cellular level. Currently, there is no consensus regarding parameters that will impact cellular response. Various conditions have been described for 1) preparation methods of ST extracts 2) dosage in cell culture or 3) detailed analyses of the ST preparation that is used for the treatment of cells. Hence, we have evaluated methods for preparation and analysis of ST extracts using 2S3 reference smokeless tobacco. Ten percent (w/v) preparations of ST were extracted in DMSO (ST/DMSO) or complete artificial saliva with enzymes (ST/CAS) for up to 24 h, and analyzed for the presence of nicotine, 21 polycyclic aromatic hydrocarbons (PAHs) and four tobacco specific nitrosamines (TSNAs). Nicotine extraction efficiency was ~90% under all conditions. While PAHs were extracted equally in DMSO at 2 vs. 24 hours, their extraction was found to be generally higher in DMSO compared to ST/CAS. TSNA extraction efficiency was equivalent for both 2 and 24 h ST/DMSO. However, TSNAs were significantly elevated in the 24 h ST/CAS-extracted sample, compared to the 2 h ST/CAS-extracted sample. We hypothesize that the increase in TSNA levels in the 24 h ST/CAS extractions is artifactual due to microbial action occurring during sample preparation. A time course of ST/CAS extracts prepared with and without antibiotics was performed. Nicotine and PAH levels were unaltered by the presence of antibiotics. Significantly, antibiotics prevented increases over time in the levels of microbes, TSNAs and nitrite/nitrate ratio. Thus, for extraction times greater than 2 h in physiologically relevant aqueous buffers such as ST/CAS, consideration must be given to potential microbial-driven artifact formation (e.g. TSNAs) which may skew biological assay results. When conducting extractions longer than 2 h, the addition of an antibiotic, or other appropriate mitigant is recommended, as necessary, to minimize artifact formation.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST05

Estimating the removal efficiency of vapor-phase phenol by cellulose acetate filters.

Filters made with cellulose acetate tow are known to selectively remove certain components from cigarette smoke. In a 2003 CORESTA paper, it was shown that selective removal could be related to a component's solubility parameters and vaporization rate. While useful for identifying the potential for

selective removal of specific smoke components, consideration of solubility parameters and vaporization rate alone do not provide insight into several important aspects of filter performance such as how selectivity will be influenced by smoking protocol or filter ventilation, or how selectivity will change on a per-puff basis. Typically, measured removal efficiencies combine the effects of particulate-phase removal, vapor-phase removal, and the dynamic partitioning of the smoke component between phases. In this work, a mathematical model has been developed that incorporates the partitioning between phases and separate terms for removal efficiency of the two phases. Application of the model to experimental data enables estimation of the portion of a component in the vapor phase and the vapor-phase removal efficiency separate from the particulate-phase efficiency. For phenol, the results indicate that approximately 15% of smoke phenol is in the vapor phase, that migration between phases is very rapid relative to the filter residence time, and that the vapor-phase removal efficiency by cellulose acetate is greater than 99%. With a few key assumptions, this approach should be applicable to any semi-volatile smoke component and could lead to a better understanding of selective filtration.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST06

Modified cigarettes. Evaluating the effect of charcoal filters and a filter with a porphyrin, in intensive smoking conditions regarding the content in cigarette smoke of nicotine, tar, CO and PHAs yield.

The composition of cigarette filters is one of the factors we can manipulate to control smoke yield. In this work we compared the TPM, nicotine, carbon monoxide and 13 PHAs (including benzo[a]pyrene) emissions of cigarettes from three different blends of tobacco with three different type of filters each: plain cellulose acetate filter (AC), combined cellulose acetate/activated carbon filter (CA) and a filter composed of cellulose acetate and paper treated with porphyrin (PO). An American blend cigarette, an English type cigarette and a dark tobacco cigarette with; AC filter, CA filter and PO filter were tested under Canadian intensive machine smoking conditions by triplicate. PHAs were analyzed by GC/MS/MS. They were extracted from the Cambridge filters of the smoking machine with 20 ml of hexane:dicloromethane (85:15), shaken and ultrasonicated 1h, injected directly in the GC/MS/MS (IonTrap Varian-Saturn 2100) and quantified with GC-EI-MS/MS.

PO filters lowered the TPM yield in all types of cigarettes (20% - 40%) without reducing the levels of nicotine, carbon monoxide or PHAs content, including the benzo[a]pyrene. CA filter did not reduce the content of any of the parameters determined.

The ineffectiveness of the CA filter and the PO filter in the cigarettes tested may be due to saturation of the active particles with the intensive smoking regimen. PO filter manufacturers claim that these kinds of filters remove a significant portion of mutagens and carcinogens from cigarette smoke, but we found no reduction in the levels of PHAs using the Canadian intensive smoking method.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. PT01

A drying model of tobacco stem expanding in air flow.

A drying model was developed to simulate changes in the water content and the temperature of a cylindrical plant material, tobacco stem, which expands in air flow mixed with or without superheated steam. The model is characterized as follows: (1) mass and heat transfers in the stem are described by

one-dimensional diffusion based on conservation laws; (2) adsorption equilibrium of water is always achieved at a solid-gas interface, so that the internal movement of water is regulated as a rate-limiting step; and (3) transfer phenomena in the expanding diffusion field are simplified by defining the expanded maximum radius as a diffusion length. The curves of water content and temperature calculated by the model were in agreement with each experimental value under various drying conditions: air temperature of 373 to 473 K and flow rate of 10 to 20 m/s. The model also represented drastic rises in temperature caused by condensation heat of water vapour in the initial drying stage. It was therefore judged that the model has validity and can be applied to estimation of the drying curves in expanding diffusion systems for tobacco stems.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT05

Urinary metabolome: Feasibility study on volatiles in urine of smokers and nonsmokers.

The urinary metabolome represents the entity of all 'small' compounds (molecular weight < 1000 Da) in this biological matrix. The metabolites can be of both endogenous and exogenous origin. Differences in the metabolome between well defined groups of individuals allow the identification of biomarkers of exposure and effect, responsible for these differences. Since there is no single analytical method, which allows the assessment of the entire metabolome, we decided to start our investigations with a manageable part of the urinary metabolome, namely the volatile compounds. As an analytical platform, we used headspace-solid-phase-microextraction (HS-SPME)-GC-MS. Forty (40) urine samples (derived from 20 smokers, 20 nonsmokers) were analyzed by this method under acidic and basic conditions. Peak finding and alignment procedures yielded in total about 1000 signals (mass fragments), characterized by m/z-values, retention times and intensities. Statistical methods such as t-test and partial least square discriminant analysis (PLS-DA) of the data set revealed significant differences between the groups of smokers and nonsmokers, which were basically caused by 100 - 150 signals. About 40 compounds, mostly smoke components and flavouring agents, meaningful for the observed differences, were identified by comparisons to reference mass spectra in relevant databases.

Taken together, the results of our feasibility study demonstrate that metabolomic investigations are potentially powerful tools for identifying smoking- and tobacco use-related biomarkers of exposure and effect.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT14

Research on the smoke aerosols size distribution of sidestream smoke.

Five cigarette samples of different types and seven cigarette samples of the same tobacco leaf but different cigarette making materials were smoked under the ISO3308:2000 standard smoking conditions. Sidestream cigarette smoke (SCS) was collected by fishtail cover and was diluted at 15-30 times online. The aerosol size distribution in SCS was determined by Eelectronic Low-Pressure Impacter with measuring range of 0.007-9.90 μm. The difference of aerosol size distribution in SCS among samples was analyzed and was compared with the results of mainstream cigarette smoke (MCS). The results showed that: (1) The RSD of repeatability experiment of the same cigarette sample was 1.71%. This showed that the aerosol size distribution of SCS could be measured accurately in the given conditions; (2) There was no obvious difference of aerosol size distribution of SCS among different cigarette samples; (3) The number of aerosol particles of the cigarette smouldering process in SCS was 80%-85% of the total number of particles. This indicated that the smouldering process was responsible for SCS production; (4) The Aerosol particles' total number and

total mass value of SCS were respectively 38-149 times and 1.2-2 times that of those of MCS. This result explained that the proportion of SCS was higher than those of MCS in environmental tobacco smoke (ETS), especially the small particles.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST11

Determination of cadmium transfer rate from the tobacco to cigarette smoke.

Heavy metals, such as chromium, nickel, arsenic, cadmium and lead, are present in tobacco smoke and are known to be toxic carcinogenic compounds to the human body.

In this study, the concentration of cadmium in Ky3R4F reference cigarette and two commercial cigarettes was analyzed by inductively coupled plasma mass spectrometry. Each cigarette sample was separated to tobacco filler, filter, cigarette paper and then analyzed and the concentration of cadmium was determined. The concentration of cadmium in mainstream smoke, ash and butt were also analyzed after smoking under ISO smoking conditions.

The recovery and repeatability of the analysis method were evaluated by using results obtained from Ky3R4F reference cigarette analysis. Recovery of the samples such as filler, filter, ash, butt and mainstream smoke showed values of 90~110%. All samples were analyzed four times and showed stable CV values of less than 15%. Cadmium concentration of samples before smoking showed 0.6~2 μg/g in tobacco filler and 0.01~0.04 μg/g in filter and cigarette paper. After smoking, cadmium concentration of samples showed 0.1~0.4 μg/g in ash, 0.06~0.2 μg/g in butt and 2~50 ng/cig in mainstream smoke. The transfer rate of cadmium from cigarette to mainstream smoke, butt and ash were 0.2~5%, 5~17% and 15~30%, respectively. We estimated that 45~60% of the cadmium in the cigarette may be present in the sidestream smoke.

The cadmium concentration transferred from tobacco filler to smoke was found at the highest level in sidestream smoke and the lowest in mainstream smoke. Through the results, we can try to minimize exposure to toxic substances for public health by understanding heavy metal transfer from cigarette to smoke. These results may be used in cigarette development to reduce harmful substances.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SS08

A model for the respiratory retention of compounds found in cigarette smoke.

For the risk assessment of cigarette smoke, it is desirable to have a reliable estimate of the mass of chemical compounds retained in the body during smoking. Filter studies can provide reliable estimates of mouth exposure to compounds in cigarette smoke, but do not account for mouth spill and respiratory retention. Urinary biomarkers can provide the relative uptake of certain compounds when comparing products, but generally do not provide absolute uptake values. Nicotine is the exception since the multiple urinary biomarkers currently measured can account for over 90% of the nicotine deposited in the body. In addition, deposition information may be desired for compounds for which no biomarker has been developed. Knowledge of the respiratory retention of smoke compounds allows mouth exposure to be converted to a more realistic measure of exposure although mouth spill is still neglected. Fortunately, a number of papers have been published that report respiratory retention. Data from multiple studies have been combined and respiratory retention has been determined as a function of vapour pressure. Averaging data from multiple studies reduces both subject and method variability. A plot of the average respiratory retention versus the log (vapour pressure at 20-25 °C)

gives a sigmoid shape with three distinct regions. Compounds with vapour pressure greater than 10^{-4} pascal (Pa) generally have respiratory retentions of 90% or greater. Compounds with vapour pressure less than 10^{-9} Pa, generally have respiratory retentions of 60% or less. A transition region lies between these ranges. Solanesol, with a vapour pressure of about 10^{-20} Pa is generally assumed to represent smoke particulate deposition. Solanesol retention increases as both inhalation volume and lung exposure time increase. A model for solanesol retention versus these variables is also presented.

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An improved HPLC method for investigating selective filtration.

Selective filtration is an important part of optimizing cigarette filter performance to meet potential regulatory requirements. Phenolic compounds are frequently used for selective filtration studies because there is general interest in enhancing their removal. Since they are known to be selectively removed by cellulose acetate filters, they provide a useful model for semi-volatile filtration mechanisms.

For such studies at Eastman, phenolic compounds are determined by HPLC after extraction of Cambridge pads or filters in an alcohol solution. Direct analysis of extracts enables the phenolic compounds to be determined from the same smoking session as used to determine NFDPM, nicotine, and CO. Efficient extraction of cellulose acetate filters enables the direct determination of filtration efficiency for these compounds. Recent improvements to the method retains these advantages while improving resolution of the LC peaks and adding additional compounds which can be measured simultaneously. Conveniently including additional smoke components enhances the potential learnings from a selective filtration study.

Among the added compounds are some that are known to be non-volatile, particulate-phase smoke components. Selective filtration is usually indicated by comparing the removal efficiency of a specific smoke component to the removal efficiency for NFDPM. However, NFDPM contains many semi-volatile smoke components that may also be influenced by the factors being investigated in a selective filtration study. The availability of a particulate phase smoke component offers a more appropriate reference for understanding the mechanisms of selective filtration.

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Preparation of microporous-mesoporous composite material for removal of harmful components in cigarette mainstream smoke.

Filter additives, with specific structures, act as adsorbent of cigarette mainstream smoke, providing a solution for reducing the deliveries of harmful components. However, the molecular size of harmful components in cigarette smoke differ from each other, it is difficult to adsorb a variety of harmful components at one time using single pore structure adsorbents. Because of this, a novel type of multiporous filter additive, microporous-mesoporous composite material (MMM), has been designed for harmful components reduction in cigarette mainstream smoke based on TEOS with CTAB and TPABr used as templates, through simultaneous crystallization. The as-made MMM was characterized by N_2 adsorption/desorption, XRD and FT-IR. The results show that the MMM has composite pore structure combining both microporous and mesoporous, and the pore size is mainly located at 0.54 nm, 1.4 nm, 2.8 nm and 3.7 nm. The specific surface area of the MMM is 361.3 m^2g^{-1} with a pore volume of 0.347 cm^3g^{-1}, in which the microporous volume and mesoporous volume are

0.112 cm^3g^{-1} and 0.235 cm^3g^{-1}, respectively. In addition, the property of the MMM to remove harmful components in mainstream smoke has been investigated. Comparing with the reference cigarette (without MMM in filter), the deliveries of some harmful components in mainstream smoke of experimental cigarette (with MMM in filter) decrease in varying degrees, in which the deliveries of HCN, NNK and phenol are reduced by 15%, 24% and 43%, respectively. The high reductions of harmful components are likely due to the multiporous structure of MMM, showing that the MMM is a promising filter additive.

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The role of cigarette smoke constituents in oxidative modification by aqueous extracts of mainstream cigarette smoke. Part II: LDL lipid peroxidation.

Oxidative stress is a state of imbalance between reactive oxygen species (ROS), and the ability of the organism to defend against them, leading to progressive oxidative damage. ROS causes oxidation of proteins, DNA and lipids, which may cause direct tissue injury or induce a variety of cellular responses. Low-density lipoprotein (LDL) lipid peroxidation is one of the markers for oxidative stress. This study aimed to determine the mainstream cigarette smoke constituents that participate in LDL lipid peroxidation in *in vitro* experiments.

Effect of gas vapour phase-bubbled phosphate buffered saline (GVP-PBS) on LDL lipid peroxidation was estimated to account for approximately 80 percent of the effect of whole smoke-bubbled PBS (WS-PBS) in the experiment with a reference cigarette, 3R4F. Also, benzo[a]pyrene, phenols in the particulate phase (PP) and carbonyls in GVP were implied as inducers of LDL lipid peroxidation by correlative analysis using 9 different sample cigarettes. Exposure to each of benzo[a]pyrene or phenol compounds in the amount equivalent to that in WS-PBS of 3R4F revealed none of the compounds induced LDL lipid peroxidation. However, the effect of a mixture of phenols on LDL lipid peroxidation was estimated to account for only about 3 percent of the effect of WS. Of the carbonyls tested, only acrolein induced LDL lipid peroxidation at almost 1.5 times in comparison with the effect of WS. Effect of GVP-PBS with acrolein in the amount equivalent to that in WS-PBS of 3R4F was less than the sum of the individual effect of GVP-PBS and acrolein.

In conclusion, GVP is more involved in the induction of LDL lipid peroxidation than PP. And, acrolein accounts for much of the effect of GVP on LDL lipid peroxidation, and its effect could be suppressed by other chemical components.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT34

Synthesis of vitamin E succinate and its conversion to vitamin E in cigarette.

It was reported that the consumption of vitamin E of a smoker is higher than that of a non-smoker by about 30%. In order to supplement vitamin E and improve cigarette taste, vitamin E succinate was synthesized from vitamin E and succinic anhydride by using DCC (dicyclohexylcarbodiimide) as catalyzer, its purity was measured as 98% by HPLC and its structure was identified by ^{1}H NMR, ^{13}C NMR and MS. The synthesized Vitamin E succinate was added to Virginia type cigarette, and the cigarette smoke analysis results showed that vitamin E succinate could increase the content of vitamin E in cigarette smoke, which released vitamin E by pyrolytic reaction during smoking. The results of panel test also showed that vitamin E succinate could decrease the irritation and harshness of cigarette,

improve the taste and quality of cigarette, and increase the content of vitamin E in the body of smokers.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST02

Monitoring glutathione levels in H292 cells as an oxidative stress model for RTP assessment.

Oxidative stress due to elevated production and levels of cellular reactive oxygen species is an important factor in numerous human disorders. Cigarette smoke is both a source and an inducer of cellular oxidative stress and has been implicated in the development and progression of smoking-related diseases including chronic obstructive pulmonary disease, cancer and cardiovascular disease. We describe the development and utilisation of an *in vitro* oxidative stress assay in which we monitored the levels of the intracellular antioxidant glutathione (GSH) in human bronchial epithelial (H292) cells.

Methods: GSH levels were measured using the GSH Glo assay (Promega) in confluent monolayers of H292 cells. Cigarette smoke total particulate matter (TPM) from University of Kentucky 3R4F reference cigarettes was trapped on a Cambridge filter pad, eluted in DMSO and added to cell media for 1 h at 37 °C prior to measuring GSH levels.

Results: TPM caused a concentration-dependent decrease in GSH levels when compared to cells treated with media alone. We examined the variability of cellular GSH levels in response to a number of experimental factors (TPM batch, cell passage number, assay plate, intra-plate replicates and experimenter). Statistically significant variation was observed for several parameters. However, as the level of variation was less than 10% of the mean it was not deemed to be biologically significant. We also examined the effects of TPM obtained from combustible reduced toxicant prototype (RTP) cigarettes compared to that obtained from appropriately-matched commercial control cigarettes. Statistical analyses showed that when using TPM derived from RTP cigarettes, lowering of GSH levels was less apparent compared to TPM derived from control cigarettes.

Conclusion: Monitoring GSH levels *in vitro* is a relevant oxidative stress assay which demonstrates acceptable levels of data variability and is suitable for the assessment of the biological effects of RTP particulate phase smoke extracts.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT26

A routine procedure for the measurement of particles or fibres released from cigarettes or filters.

Particle or fibre release from cigarettes during smoking has been the subject of much debate in recent years. A number of studies have been carried out to quantify the number and type of particles or fibres released from cigarettes or filters during puffing. Many of these methods involve dry puffing followed by relatively complex procedures, such as scanning electron microscopy or infrared microspectrometric analysis, to identify the number and nature of the particles or fibres released. To allow screening of a wide range of products a more rapid procedure to estimate the number of particles released is required. Laser particle counters are used to monitor air quality in a range of applications and offer a rapid measure of total particle quantity.

A routine method for particle counting is described based on a modified laser particle counter and flow switching system. This method allows particles eluted from cigarettes or filters in the size range from 0.5 to 50 microns and at a range of different flow rates to be rapidly counted.

Typical data is also given from the use of the procedure to evaluate the suitability of carbon manufactured from a range of raw materials such as coconut, coal, wood and peat, in terms or particle release, for use in cigarette filters.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST08

Further investigations of temperatures and adsorption / desorption of volatile compounds in carbon filters under different smoking regimes.

Previous work on "7 mg ISO tar" cigarettes containing an activated carbon filter had shown a significant removal of volatiles in mainstream smoke under ISO smoking. Our work had also shown that high filter temperatures associated with the Canadian intense (CI) regime lead to a significant desorption of volatiles from carbon leading to an increase of mainstream smoke yield.

This study investigated filter temperatures and volatile retention under the ISO, Massachusetts, WG9 option B (WG9B) and CI smoking regimes. Temperatures were measured in the middle of the carbon section. Smoke temperatures passing through the filter, were highest for the CI and lowest for the ISO smoking regime.

Thermal desorption / gas chromatography / mass spectrometry analysis was used to monitor the relative distribution of volatiles between those adsorbed on the filter and those in the mainstream vapour phase according to the smoking regime. The experimental set-up was designed to evaluate the temperature effect on this relative distribution. A first experiment analysed the volatiles during smoking when placing the carbon section within the cigarette filter. A second experiment excluded the increase of temperature by placing the carbon section external to the filter and maintained at ambient temperature. The comparison of the resulting volatile distribution between those adsorbed in the filter and those delivered to mainstream smoke vapour phase allowed an estimation of the impact of temperature on the retention of volatiles.

In comparison with ISO and CI regimes, the other regimes induce intermediate filter temperatures and intermediate reduction of carbon filter efficiency even though puff volumes are higher in the case of the WG9B than the CI regime, showing that partial ventilation blocking still allows filter cooling. Additionally, carbon filters are a well known technology to reduce vapour phase yield under ISO smoking but for the most volatile compounds this tends to decrease during more intensive smoking especially under the CI regime.

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Contribution of polyphenolic complexes to the yield of phenols in tobacco pyrolysis.

Polyphenols in tobacco leaves, such as chlorogenic acid (CGA) and rutin, are major precursors of phenols in tobacco smoke, and it is known that the polyphenols form polyphenolic complexes with other tobacco leaf components, such as amino acids and proteins during curing. However, the contribution of the polyphenolic complexes to the yield of phenols in tobacco smoke is not clear. The objective of this study is to evaluate the contribution of the polyphenolic complexes to the yield of

phenols (hydroquinone, resorcinol, catechol, phenol, *o*-, *m*- and *p*-cresols) in tobacco pyrolysis. Flue-cured tobacco was extracted with a H_2O/MeOH (1/1) mixed solvent, and the extract was subjected to sequential ultrafiltration (10, 5 and 1 kDa) followed by a polyvinylpolypyrrolidone (PVPP) treatment which can remove low molecular weight polyphenols selectively. Tobacco extract residue was divided equally, and the treated solutions were restored to each tobacco residue. Polyphenols (CGA and rutin) and total polyphenols in prepared tobacco samples were analyzed by high performance liquid chromatography (HPLC) and a colorimetric method using Folin-Ciocalteu reagent, respectively. The tobacco samples were also pyrolyzed, and the phenols in smoke were determined by HPLC. As a result, it was found that ultrafiltration of 10 and 5 kDa did not substantially affect the total polyphenol and polyphenol content in the tobacco samples, but ultrafiltration of 1 kDa decreased the total polyphenol content by 23% despite little change in CGA and rutin content. As for the yield of phenols, 6 phenols except resorcinol were reduced by approximately 20% (resorcinol: 36% reduction) by ultrafiltration of up to 1 kDa. These results indicate that polyphenolic complexes ranging mainly from 1 to 5 kDa contribute significantly to the yield of phenols. The contribution of low molecular weight polyphenols which can be removed by PVPP will also be discussed.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST25

γ-irradiation of tobacco reduces cigarette smoke induced inflammatory responses.

Cigarette smoke induces inflammation and has an effect on the immune system. Toll-like receptors (TLRs) are key elements in pathogen recognition by the host immune system. Although TLRs have been intensely studied in innate immunity and infection their critical role in non-infectious challenges has only recently emerged. TLR9 has been described to be involved in the pathogenesis of several diseases and CpG, a DNA fragment, is its endogenous activator.

It has been demonstrated that cigarette smoke extract (CSE) induces CXCL8 release via TLR9 activation of human neutrophils, which was confirmed in TLR9 stably transfected HEK293 cells. Here we demonstrate that treatment of CSE with the enzyme DNase (which breaks down DNA), decreases the ability to stimulate neutrophils to release CXCL8 and MCP-1. Interestingly, CSE obtained from γ-irradiated standard cigarettes (0.1-6.4 kGray) also decreases the CXCL-8 release from several inflammatory cells. Further, CSE obtained from standard cigarettes increases the surface expression of CD54 on human neutrophils as CSE from irradiated cigarettes did not.

Exposure of mice to cigarette smoke (nose-only, side and mainstream) twice a day for 3 days (<30 min), increased the number of inflammatory cells in the lungs with standard cigarettes but not with γ-irradiated cigarettes. Moreover, γ-irradiation of cigarettes reduces the levels of double stranded and single stranded DNA levels in CSE.

In conclusion, γ-irradiation of tobacco decreases the ability to induce an inflammatory response *in vitro* and *in vivo*. These data may contribute to the development of tobacco products with a lower risk for human health.

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GCxGC-MS characterisation of tobacco extracts: Classification and factors affecting the class maps.

Comprehensive gas chromatography (GCxGC) appears as the preferred technique for the characterization of volatile compounds in tobacco samples. Indeed, GCxGC offers an enhanced separation power which results from the combination of two columns of different selectivity - the first column is usually non-polar and the second polar. The main advantage of GCxGC is to reduce the number of co-elutions and hence to provide a way to increase the capacity to discriminate a maximum of compounds. However, the huge number of peaks, and consequently of information, contained into the 2D chromatograms induces the necessity to develop a strategy to process the data automatically.

As shown in our previous work, any global comparison of GCxGC chromatograms and discrimination of tobacco classes requires several preprocessing steps such as background correction, intensity normalization, and especially time alignment, along the first or even both dimensions. After preprocessing, a correlation study enables us to locate the pixels that are discriminant for each class of samples, and to elaborate a characteristic map for each class. These maps can be seen as templates allowing the identification of areas of interest, i.e. areas corresponding to compounds over expressed or under expressed in one class as compared to all others.

Henceforth, this first step is carried out by an automatic process embedded in a user-friendly graphical interface (G.U.I.), which facilitates data visualizations and analyses. The present study focuses on the map characteristics and how they may be affected by the various sources of variability of the input data: differences of tobacco origin, sample to sample variability, extraction to extraction variability, and finally injection to injection variability. The G.U.I. provides a basis for statistical analysis useful to determine the main sources of data variation, which demonstrate its fit for purpose.

Ultimately, the variability analysis based on correlation maps, combined with mass spectrometry information, will provide a new tool to identify systematically the compounds responsible for the differences observed between different classes of samples and thus identify the corresponding chemical markers.

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Factors affecting the design of paper diffusivity measurement apparatus with particular reference to the design of transfer standards.

The measurement of CO_2 diffusion through paper substrates has been demonstrated to correlate well with the ability of a cigarette to pass the ASTME2187-04 test for ignition propensity. The design of the apparatus making such measurements must be such that a high degree of reproducibility and linearity is achieved. Comparisons have been hampered in this by a lack of a suitable transfer standard. To overcome this deficiency a laser drilled strip was developed to repeatedly mimic papers of differing diffusivity. The significant design parameters of the strip are presented.

A number of factors affect performance, a key factor being the design of the gassing head. This paper describes two alternative gassing head designs and compares their performance. Using finite element analysis of flow and practical measurements the ability of the two heads to provide linear, repeatable results on paper and standards are explored.

Recommendations are made as to the preferred geometry of the test head and further development and use of transfer standards.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT31

Mutagenic, cytotoxic, and genotoxic properties of tobacco smoke produced by little cigars available on the Canadian market.

Little cigars (also known as cigarillos) have been increasing in popularity with respect to cigarettes; but relatively little is known about the toxicology of the mainstream smoke (MSS) from little cigars. Therefore, the objective of this study was to compare the toxicological properties of the MSS (Health Canada Intensive smoking conditions) from a range of little cigar products with the toxicological properties of MSS of other smoking products such as cigarettes. Three *in vitro* assays were used to evaluate the toxicities of the MSS total particulate matter (TPM): 1) mutagenicity using Ames assay with strains TA98 and TA100 with S9 metabolic activation (+S9); 2) cytotoxicity using the Neutral Red assay with CHO cells; and 3) genotoxicity using the micronucleus assay with CHO cells and short-term exposures (3-hour ±S9). The Ames assay (TA100+S9) and the Neutral Red assay were also applied to the gas/vapour phase (GVP) of the MSS that passed through the Cambridge pad. On a per-milligram nicotine basis, which is one way of comparing toxicities of different types of tobacco products, TPM from the little cigars was more mutagenic with TA98+S9 and TA100+S9 than was the TPM from cigarettes (70.6±11.5K and 26.5±7K versus 34.4±5K and 16.1±3K revertants/mg-nicotine). A similar but less pronounced trend was noted with the micronucleus results. The GVP fractions for some little cigars were more mutagenic with TA100+S9 than were the corresponding GVP fractions from other little cigars and cigarettes, which showed very little mutagenicity. The TPM and GVP smoke fractions from the little cigars were more cytotoxic than were the corresponding smoke fractions from cigarettes (TPM IC50 1.80±0.35 versus 3.40±0.56 mg nicotine/mL, GVP IC50 3.73±0.70 versus 5.16±0.65 mg nicotine equivalent/mL). Thus, our findings support our prior work on smoke mutagenicity that showed MSS from little cigars was not less toxic than MSS from cigarettes.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPTPOST12

Studies on qualitation and quantitation of sugar components in mainstream smoke of cigarettes.

As one of the most favourite tastes, sweetness has great influence on cigarette smoke flavour. However, up to now there were few studies on relationship between sweet taste and cigarette smoke flavour. So, common sweet substances in mainstream smoke (MS), such as monosaccharides, sugar alcohols, and sweet amino acids were researched here. As has been reported, there were much less free amino acids than the other two substances. In addition, except for sweet taste, sugar alcohols give a pleasant cool and fresh sensation. Hence, monosaccharides and sugar alcohols were specially determined. Above-mentioned carbohydrates were derived with hydroxylamine hydrochloride and acetate anhydride, and then analyzed by gas chromatography-mass spectrometry (GC-MS) with CI ion source and SIM scan mode. Monosaccharides were quantitated with Metrohm ion exchange chromatography (IEC) and METROSEP Carb1 250 column, eluting with 2.0 mmol/L NaOH and 0.5 mmol/L NaAc at flow rate 1.00 mL/min, and post-column deriving with 0.1 mol/L NaOH at flow rate 0.40 mL/min. In MS, seven aldoses (ribose, rhamnose, arabinose, lyxose, xylose, glucose, galactose) and seven sugar alcohols (erythritol, ribitol, arabitol, xylitol, inositol, mannitol, sorbitol) were first simultaneously determined, and their ion fragmentation and retention time were analyzed. Galactose and sugar alcohols were first reported. Results indicated that the order of monosaccharide contents was fructose>glucose>arabinose>mannose>galactose>xylose, and the ranges of glucose and fructose content were about 42 μg/cig.~100 μg/cig. Concentration of total monosaccharides could reach the

threshold of sweet taste. Contents of sugar alcohols might be even much more. In cigarette smoke, glucose, fructose and sugar alcohols could have a significant contribution to sweet taste and other pleasant sensations. The results and conclusions might be of great significance to cigarette manufacturing technology under the background of reducing tar and other harmful components from cigarette.

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Influence of humidity, number of filter papers, and orientation of the filter paper on ASTM results.

The ASTM test method E-2187 specifies conditioning cigarettes and filter paper at 55 ± 5% RH and a temperature of 23 ± 3 °C for at least 24 hours prior to testing. The recent version of the ASTM test method (E2187-09) also specifies the orientation of the filter paper that the cigarette is placed on, the rough side. All LIP standards established in many countries use the ASTM test method and specify no more than 25% full length burns on 10 layers of Whatman #2 filter paper. Very little if any data is available on the influence of relative humidity on the test results and recent modification specifies the orientation of the paper filter. This paper will provide an update on the changes to the ASTM test method, explore the influence of changes in relative humidity in increments of 5% on 10 and 3 layers of filter paper, and test the impact of filter paper surface orientation. Two cigarette brands were identified to perform this study. According to the ASTM test method one of the brands gave 20% and the second 15% full length burns on 10 layers of filter paper.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT01

A strategic framework for the assessment of Potential Reduced Exposure Products.

A framework of laboratory, clinical and consumer evaluations is being developed that will allow a scientific assessment of whether use of new technologies and tobacco products is likely to result in (1) substantial reductions in exposure to tobacco smoke toxicants and (2) how such reduced exposure might relate to a reduction in risk of smoking related diseases. This PREP Assessment Framework outlines the different stages of evaluation including product stewardship of new technologies, smoke chemistries, consumer behaviour and sensory testing as an initial stage before clinical studies. These latter studies estimate any reduction in exposure to smoke toxicants and possible changes in biomarkers of effect. The significance of any biological effect from clinical studies is supplemented with a series of concurrent *in vitro* biological assays of disease processes. The assessment and proposed dossier will be in a format similar to the CTD used in pharmaceutical submissions, based on a "weight of evidence" approach. In the longer term these assessments will also include computational toxicological modelling as a means of integrating the data from the different studies. Although the results from controlled laboratory assessments and clinical studies should give some indication of individual's reduced exposure and changes in potential risk, it is anticipated that the wider population effects of these prototype products will have to be assessed in larger scale studies, such as limited "blind" test markets with market surveillance.

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The effect of puff volume on *in vitro* toxicity of mainstream cigarette smoke.

Different smoking parameters may be important with regard to the toxicological assessment of cigarette smoke. These include puff frequency, puff volume, and blocking of filter ventilation. Hitherto, published papers primarily focus on mutagenicity and toxicity of smoke condensate. The aim of this study was to investigate the effects of varying machine smoking parameters on the *in vitro* toxicity of cigarette smoke, in particular whole smoke and vapour phase toxicity. Puff volume and filter ventilation are the main parameters which influence tobacco combustion and the resulting chemical composition of smoke. Higher flows of air through the lit cigarette result in a higher burning temperature of tobacco. Higher puff volumes and lower ventilation increase the water level in condensate. These effects should be considered when assessing smoke toxicity.

An unventilated and four differently ventilated American Blend style cigarettes were used in this study, and three different puff volumes (35, 45 and 55 mL) were applied. The cigarettes were smoked with 2 puffs per minute (puff duration: 2 sec). Human liver Hep-G2 cells were treated in two different ways to assess cigarette whole and vapour phase toxicity; testing (i) the culture medium extracts of mainstream smoke or ii) the freshly generated smoke aerosol itself are possible methodologies. In case of bubbled medium, particulate matter and some selected vapour phase compounds are trapped only partly.

Due to the high transfer of toxic volatile compounds into bubbled medium, whole smoke extract toxicity is mainly dominated by vapour phase. Consequently, the relative contribution of vapour phase to whole smoke toxicity can be determined easily.

The 'Burghart smoke aerosol cell exposure apparatus' was used for the toxicity assessment of freshly generated smoke. This exposure apparatus allows direct contact of smoke in ambient air to the cells directly after a puff. The puff-by-puff treatment of 96 multiwell plates allows selective toxicity testing of first and last puffs of a cigarette.

The data show higher cigarette smoke toxicity with increasing puff volume. The relative cigarette specific toxicities (considering Dry Particulate Matter yield) show only moderate differences. With regard to whole smoke cytotoxicity, the contribution of vapour phase depends on puff volume and level of filter ventilation.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. SSPT04

Differential exposure biomarker levels among non consumers of tobacco, consumers of oral tobacco products and cigarette smokers in the US National Health & Nutrition Examination Survey 1999-2008.

Consumer exposure to smokeless tobacco (SLT) and cigarette mainstream smoke (MSS) constituents depends primarily on the frequency, intensity, and duration of the consumer's exposure and on the chemical composition of the MSS or SLT product used. The complexity of estimating consumer exposure to tobacco-related chemicals is compounded by large inter- and intra-individual variability in usage patterns, constituent concentration variability within and between product types and configurations, and non-tobacco-related chemical exposures (e.g., dietary, environmental, occupational). Biomarkers can inform the exposure assessment process, as they provide direct evidence of both external exposure and uptake into the body. The National Health and Nutrition Examination Survey (NHANES) is a publicly available source of survey data representative of the civilian, non-institutionalized population of the United States. Consumption of tobacco or nicotine

replacement therapy (NRT) products is among the lifestyle data collected, and biological samples (i.e., whole blood, serum, and urine) are also collected for a broad range of exposure biomarker analyses. In this study, exposure biomarkers were evaluated for selected environmental chemicals that have also been identified in SLT and/or MSS. These include certain volatile organic compounds, halogenated aromatic hydrocarbons (HAHs), polycyclic aromatic hydrocarbons (PAHs), acrylamide, and metals. Adjusting for age, gender, ethnicity, poverty-income ratio, and body mass index, the results suggest that, with the exception of some HAHs, exposure biomarker levels of analytes evaluated are significantly lower in SLT consumers (i.e., chew and snuff) than levels reported in cigarette smokers. Further, with the exception of some PAHs, exposure biomarker levels of analytes in SLT consumers are not significantly different than levels in non-consumers of tobacco or NRT products. These results strengthen the scientific basis for tobacco harm reduction through migration and are essential to informing risk assessment and regulatory processes.

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Analysis of principal and minor alkaloids in tobacco.

Nicotine is the principal alkaloid in tobacco, and nornicotine, anabasine, anatabine, myosmine, nicotyrine and 2,3'-dipyridyl are the minor alkaloids in tobacco. Because of their importance, developing a fast and accurate quantitative method to analyse tobacco alkaloids is necessary. A method for the determination of these seven alkaloids in tobacco was established based on heart-cutting multidimensional gas chromatography (MDGC). The alkaloids were extracted by 10%NaOH/tert-butyl methyl ether (TBME) and determined by MDGC with flame ionization detection (FID). The separation of alkaloids and the reliability of quantitative results were improved significantly by MDGC. The recoveries for the alkaloids ranged from 90% to 110% and relative standard deviation based on five individual preparations were lower than 5%. Tobaccos of four different types were analyzed, including 86 flue-cured tobaccos, 12 Burley tobaccos, 17 yellow sun-cured tobaccos and 30 Oriental tobaccos. The results showed that the contents of alkaloids varied with tobacco types, which in the order of contents of total alkaloids, nornicotine and anatabine were Burley>yellow sun-cured>flue-cured>Oriental. The contents of nicotine, anabasine, nicotyrine and 2,3'-dipyridyl were similar in Burley and yellow sun-cured tobaccos, which were higher than those in flue-cured and Oriental tobaccos. Among the minor alkaloids, nornicotine was the highest in Burley tobacco, anatabine highest in yellow sun-cured and flue-cured tobaccos, while both nornicotine and anatabine were the highest in Oriental tobacco. The ratio of nornicotine to anatabine in yellow sun-cured tobacco was close to that in flue-cured tobacco. Myosmine is positively correlated with nornicotine in all types but Oriental tobacco. The results also showed that alkaloid content significantly related with tobacco style and quality.

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CORESTA Congress, Edinburgh, 2010, Smoke Science/Product Technology Groups, abstr. PT11

A novel polycationic composite material with moisture retention ability applied to reduce phenolic compounds in cigarette smoke.

Phenolic compounds, such as phenol, cresols, catechol and resorcinol, are included in the so-called "Hoffmann" list of the most hazardous compounds found in cigarette smoke. It is considered desirable to potentially reduce the delivery of these compounds to the smoker. Therefore, a novel polycationic composite material with moisture retention ability was developed and applied to reduce the phenolic

compounds in cigarette smoke. The hydrophilic polycationic material, poly (vinyl galactose ester-co-methacryloxyethyl trimethylammonium chloride) was synthesized and assembled with sodium alginate on activated carbon by layer-by-layer assembly technique to construct a moisture retention film. The formation of the multilayer was followed by the determination of water contact angles and the composite material with the multilayer was characterized by Fourier transform infrared spectroscopy, scanning electron microscopy and thermogravimetric analysis. The moisture in the composite material could be controlled and retained in the range of 0 to 35%; when temperature increased to 100 °C, only less than 5% of moisture was lost. When relative humidity was higher than 50%, moisture could be hardly lost. Furthermore, the composite materials added to cigarette filter could reduce more than 70% of seven phenolic compounds in mainstream cigarette smoke.

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Zirconia modified mesoporous silica monolith: A new adsorptive material to reduce the tobacco specific nitrosamines (TSNA) content of mainstream smoke.

Tobacco smoke contains more than 4000 individual components in vapour phase and particulate phase. Therefore it is difficult to selectively capture the target carcinogens such as nitrosamines among varied compounds. Tobacco specific nitrosamines (TSNA) exist in the particulate phase in smoke, and the size of these particles are usually in the μm range, exceeding the pore size of zeolites and mesoporous silica materials that is in the range of nm. Thus, new mesoporous adsorbent with the net-like morphology and modification of zirconia was developed; the former enables the material to intercept the particulates with μm size, like the fishing-net to hold up fish, while the latter realizes the selective adsorption of TSNA through electrostatic interaction toward nitrosamines. Nitrosamines are the compounds with a characteristic group of N-NO, and this N-NO group is easily attracted by the cations inside the adsorbent therefore the group and the whole nitrosamine molecule moves toward the channel of the mesoporous adsorbent. When the sample is put in the filter of cigarette with proper path length, the pressure drop will keep unchanged. The monolith mesoporous adsorbents differ from either cellulose acetate or activated carbon. They are molecular sieves hence they can possess the unique selective adsorption function to filter the particles in smoke and to adsorb the TSNA adhered on these particles. For instance, one sample with the addition amount of 30 mg per filter reduces 17% of NNN and 14% of NNK but only 2% of TPM. Another sample with the amount of 25 mg per filter reduces 19% of NNN and 21% of NNK as well as the TPM reduction of 15% in tests.

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