ABSTRACTS OF PRESENTATIONS MADE AT THE
2008 CORESTA CONGRESS IN SHANGHAI, CHINA
SMOKE SCIENCE AND PRODUCT TECHNOLOGY

(in alphabetical order of first authors)

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Results of Yield In-Use cross laboratory proficiency testing.

Human smoke tar and nicotine yields or Yield In-Use (YIU) can be estimated by the analysis of mouth end sections of filters collected from smokers, followed by comparison of data with results from machine smoking (calibration). British American Tobacco (BAT) has used this technique in a number of countries to determine consumers' In-Use yields. The technique as developed by BAT Group R&D laboratories has been transferred to other BAT laboratories or contract laboratories. Although the principle of the method is the same across all laboratories, different types of smoking engines and analytical instruments are used. In 2007 a cross laboratory proficiency test was conducted with five laboratories to obtain a measure of inter-laboratory reproducibility.

To compare results, test filter tips were produced by machine smoking, in one laboratory, three products at three regimes to produce nine 'levels' of test tips. The test tips were randomized for each level to ensure homogenous samples, before being sent to each laboratory along with cigarettes for calibration smoking. Each laboratory produced calibration curves for each product by machine smoking and analysed the test tip extracts for nicotine and UV absorbance. The estimated tar and nicotine in-use were calculated and returned in a standard format for statistical analysis.

For estimated tar yields, the reproducibility for each 'level' was found to be between 7.2% and 18.7%, but generally the results were within 15%. For estimated nicotine yields, the reproducibility was found to be between 6.2% and 14.7% but generally the results were within 10%. Work is currently being undertaken to investigate the principal causes of this variation and improvements will be incorporated into the YIU methodology. Further cross laboratory proficiency tests will then be conducted.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST02

A highly sensitive and specific analytical procedure for the determination of a tobacco specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in human urine using molecular imprinted polymer extraction, hydrophilic interaction liquid chromatography and mass spectrometric detection.

This paper will discuss a highly sensitive and specific analytical procedure for the determination of 4-(methylnitrosamino)-1-(3−pyridyl)-1-butanol (NNAL), a tobacco specific nitrosamine, in human urine using a combination of molecular imprinted polymers (MIPSs) solid phase extraction, hydrophilic interaction liquid chromatography (HILIC) and triple quadrupole tandem mass-spectrometric detection.

MIPs are engineered cross-linked polymers that can exhibit high affinity and selectivity towards a single compound. MIPs particles have a selective synthetic recognition site (or imprint) that is sterically and chemically complementary to a particular analyte. MIPs are able to bind specifically to trace level analytes in complex matrices (such as urine) in the presence of large excesses of other compounds that have similar physico-chemical properties.

HILIC uses a polar stationary phase such as silica and a less polar solvent as the mobile phase, typically acetonitrile. Elution is achieved by increasing the aqueous portion. HILIC gives good retention of polar bases such as NNAL. Two modes of retention mechanism can be observed:

(i) partitioning of the analyte between a water layer absorbed to the surface of a stationary phase and the mobile phase
(ii) cation exchange of charged polar analytes with charged surface silanol groups.

Triple quadrupole tandem mass spectrometric detection was used with Turbo Ionspray in positive mode. A mass transition of 210.3 to 180.1 Da was monitored.

The glucuronide (NNAL-N-ß-D-glucuronide) was determined by liberation of NNAL using enzymatic deconjugation (ß-glucuronidase from *Escherichia coli*) and quantified versus an NNAL calibration curve.

A quantitative analytical procedure with sensitivity to 5 pg/mL using a sample volume of 500 µL and linearity to 1000 pg/mL was successfully developed and validated.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST22

A snus extraction methodology to permit thorough *in vitro* toxicology testing.

The *in vitro* toxicology testing of snus presents differing challenges to those encountered when testing combusted tobacco products, principally as the material to be assessed is insoluble (but absorbent) plant matter. By applying certain modifications to ISO guidelines that exist (for the biological evaluation of insoluble medical devices), Snus extracts can be prepared which are suitable for use in various *in vitro* genotoxicity and cytotoxicity assays.

The objective of the experimentation was to establish an extraction methodology for snus samples, that would permit a robust safety assessment in the Ames, Mouse Lymphoma, *in vitro* Micronucleus and Neutral Red Uptake assays.

Snus samples with varying nicotine and water contents were extracted for 24 hours at 37 °C in both water and dimethyl sulphoxide (DMSO), at varying concentrations ranging from 200 to 500 mg (equivalent) per mL. At 500 mg (equivalent) per mL, the snus absorbed up to 85% of the extraction vehicle, making extraction at any higher concentration impractical. By measuring the nicotine content of the snus extracts, the percentage recovery of nicotine from the snus could be determined. Nicotine content was measured as 82-86% for aqueous snus extracts at 200 mg (equivalent) per mL, and at 73% for extracts at 500 mg (equivalent) per mL.

A 500 mg (equivalent) per mL extract was therefore considered to be the highest extraction concentration that could practicably be extracted from snus or moist snuff. Subsequent *in vitro* genotoxicity assays performed using snus extracts at this concentration resulted in biological dose limiting effects in some cases, and/or achieved (or exceeded) exposure concentrations required in the standard regulatory guidelines applicable for these assays, and was therefore acceptable for safety assessment.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST03

Comparison and optimisation of methodologies for the detection of urinary mutagens from donor smokers.

The presence of mutagens in cigarette smokers' is widely documented, and urine mutagenicity is recognised as a useful biomarker of exposure. Extraction and concentration techniques are required for evaluating mutagens in smokers' urine by bacterial mutation (Ames) assay. Historically this has most frequently been conducted using XAD-2 adsorption columns, but this methodology can be subject to variability in extraction efficiency if there is any inconsistency in the quantity and density of the resin packed into the columns.

The objective of the experimentation presented was to establish whether there were any discernible differences between urine extracts prepared using the two different sorbents, by assessing the relative mutagenic responses obtained, and whether these mutagenic responses could be enhanced by optimising several bacterial mutation assay conditions.

Direct comparisons were made between the mutagenicity of smokers' urine extracted using in-house prepared XAD-2 columns and those extracted using pre-packed commercially available octadecyl (C18) columns. When pairs of identical samples of smokers' urine were extracted using the two methodologies, analogous responses were obtained with each pair of extracts following bacterial mutation assay. It was therefore concluded that equivalent extracts from smokers' urine were obtained whether C18 or XAD-2 columns were used.

Further modification of the microsuspension bacterial mutation assay commonly used for mutagenicity assessment of urine extracts was conducted. This involved reduction of the phosphate buffer concentration, increasing the sample volume, increasing the pre-incubation vessel volume and increasing incubation shaking speed. By optimising these factors, increases in the sensitivity of the assay (enhanced mutagenic responses) were achieved, as demonstrated by revertant numbers as much as 1.8 fold and 1.5 fold above those achieved without the further modifications for strains TA98 and YG1024 respectively.

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An improved headspace solid-phase micro-extraction method for the analysis of "free-base" nicotine in particulate phase of mainstream cigarette smoke.

The content of "free-base nicotine" in cigarette smoke is a controversial subject, partly due to methodological issues. In this investigation, an improved method to measure volatile nicotine ("free-base nicotine") using headspace solid-phase micro-extraction (HS-SPME), combined with gas chromatography/mass spectrometry (GC/MS) was developed and validated for this purpose. Cigarette smoke particulate phase (PP) was collected onto a 44 mm glass fiber filter pad. The pad was then cut in half using one section for the determination of total nicotine and water contents. The other was transferred into a 20 mL amber glass vial for analysis by HS-SPME. The following factors were found to have a significant impact on the results: SPME fiber type, pre-equilibrium time before HS-SPME, extraction time and temperature, PP water content, and the solvent used for the preparation of standards. In this study, a PDMS/DVB fiber was found to provide sensitivity and peak shape superior to that obtained using a Carboxen/PDMS fiber. "Free-base nicotine" reached absorption equilibrium onto the fiber in less than 5 minutes, but more than 2 hours were required in order to obtain partitioning equilibrium between PP and the headspace. Control of the extraction temperature was critical for the reproducibility of analysis. Also, it was found that the partitioning into the headspace and extractability of "free-base nicotine" was dependent upon the PP water content. This necessitated the introduction of a moisture correction factor based on an experimentally determined reciprocal model. Under ISO smoking conditions method characteristics were: limit of quantification - 1.32 µg/cig, with a recovery from fortified solutions of 96.4%, and a coefficient of variation (CV) of 9.8%. Results for reference cigarettes (average, CV) were: Canadian flue-cured monitor - 34.6 µg/cig, 14% and Kentucky Reference Cigarette 2R4F - 36.1 µg/cig, 11%.

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Determination of multiple organochlorine, pyrethroids and 2,6-dinitroaniline pesticide residues in tobacco.

A method of determining the residues of forty-four pesticides, including 28 organochlorine, 10 pyrethroid and 6 2,6-dinitroaniline pesticides, in tobacco by accelerated solvent extraction (ASE) and gas chromatography was presented. Tobacco sample was mixed with Florisil and extracted with ASE using n-hexane and acetone (1:1, V/V), the extract was concentrated and then determined by gas chromatography with electron capture detector, mirex was used as an internal standard. Good recovery (74.7%-105%) and repeatability (RSD < 10%) for analytes can be achieved. This method proved to be fast, accurate, more automated, and consumed less reagent.

Key words: organochlorine pesticide, pyrethroid pesticide, 2,6-Dinitroaniline pesticide, ASE, gas chromatography, tobacco

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT09

A multiple covariance based method to explore relationships between Hoffmann analytes and several groups of physical and chemical cigarette parameters.

In this work, we studied 28 commercial Virginia and US blends described using various thematic variable groups. Our purpose was to explore the possibility of an explanatory linear model connecting structural dimensions of these groups. A group containing 14 smoke Hoffmann constituents is assumed to depend on 29 chemical tobacco variables, 10 product physical variables, and 4 major smoke compounds (Tar, Nicotine, Carbon monoxide, Water).

To solve this problem, we used a multi-array exploration technique: Structural Equation Exploratory Regression (SEER). The purpose of SEER is related to that of more classical methods such as PLS Path Modeling, Multi-block PLS and LISREL (References). But there are fundamental differences in approach and computation. SEER is carried out as described below.

First, one must define a thematic partitioning of explanatory variables. This partition is generally based on expert knowledge; yet, in practice, one may start with a basic conceptual model (for example chemical, physical and TCN) and refine it gradually by taking into account the empirical findings provided by former SEER-estimations (we have chosen this latter attitude to illustrate the flexibility of SEER). Second, one uses a multiple covariance criterion extending that of PLS regression to simultaneously extract a small number of structural dimensions in thematic groups (for the sake of robustness) and investigate the linear model linking them. The use of a model that does not compel structural dimensions to be orthogonal between themes allows better use of the conceptual and statistical complementarity of groups, yielding a more realistic and interpretable model than PLSR. A series of graphics help interpret the model, and classical goodness of fit indicators help validate it.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT52

The application of the composite porous materials to reduce the harmful compounds in mainstream smoke.

In order to reduce the harmful compounds in cigarette smoke, two types of composite porous materials are synthesized. Based on mesoporous materials, SBA-15 and MCM-41, zeolites HZSM-5 and NaY fragments are introduced into the synthetic system and assembled with mesoporous materials. These composite porous materials combine the advantages of micro- and mesoporous materials. And they exhibit higher effects on reducing the tar free radicals, TSNAs and some vapor phase compounds than activated carbon. In another way, zeolite HZSM-5 is coated into activated carbon and tailored by alkali solution, respectively. Their ability to reduce TSNAs in mainstream smoke is also obviously improved compared with activated carbon.

Key words: zeolite, mesoporous material, activated carbon, composite porous material, mainstream smoke, carcinogenic compound

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The application of different levels and types of burn additives to cigarette paper and the resultant effects on mainstream and sidestream smoke yields.

Both mainstream and sidestream smoke are of interest to cigarette designers and for regulatory bodies in various parts of the world. The role of changing cigarette paper characteristics on the yields of various smoke phases is therefore of interest to the tobacco industry.

The objective of this exercise was to undertake an experimental design on cigarette paper in which the level and type of three different potassium salts was systematically varied on a constant cigarette base paper of known basis weight, filler level and permeability. The potassium salts in question were tri-potassium citrate, potassium formate and potassium gluconate. The choice of salts was made on the basis of their corresponding decomposition temperature in comparison to cellulose. In addition their good solubility in water allowed a wide range of applications onto the base paper via a size press operation on a papermaking machine

Machine made cigarettes were produced with these papers utilising a constant cigarette construction in terms of tobacco column density, blend type, and filter format. Mainstream and sidestream smoking was undertaken and the smoke analysed for nicotine, NFDPM, carbon monoxide, and puff number, additionally sidestream carbon dioxide yields were recorded.

Compared to the burn additive free cigarette paper, mainstream yields of NFDPM and nicotine were reduced by up to 30% as were sidestream NFDPM yields by the application of the salts. Mainstream gas phase yields were also reduced, but sidestream gas phase yields were increased with increasing salt concentration, as would be expected on the basis of a constant cigarette construction being used. The effects of the various salts are considered in relation to both the cation and anion concentrations in addition to their decomposition temperature.

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The use of magnitude estimation to assess the odour and irritation of sidestream smoke - (Part 2) with the fabric method.

Sidestream smoke, which is the smoke that comes from the lit end of the cigarette, is an active area of research and development for cigarette designers. The overall objective of this research was to develop a 'fabric' methodology for the assessment of sidestream smoke odour and irritation. The method involved sidestream smoke deposited onto samples of fabric held in a perspex box. The fabric was then transferred into jars and assessed by panellists. The first stage was to monitor the consistency of sample sets and assess levels of repeatability, by analysing the headspace of samples. The second stage was to identify the optimum amount of smoke to be assessed by building a dose-response relationship between the amount of sidestream smoke and the intensity of the sensory perception (irritation and odour).

To achieve the second stage, a known reference standard (1-butanol) was used to determine the detection threshold under the conditions of the 'fabric in jar' method. This approach was carried out by preparing serial dilutions of 1-butanol. These dilutions were assessed to measure psychometric functions for the detection of odour and nose irritation. The detection threshold was obtained at the 50% chance-corrected probability point. Panellists were recruited, selected and trained according to the ISO method 8586-1. Panellists were then trained to score intensity ratings using ratio-scaling (magnitude estimation) and a standardized procedure using 1-butanol with a reference (fixed modulus).

Subsequently, panellists were trained to rate a range of sidestream smoke concentrations (reference cigarette 3R4F). The optimum sidestream concentration to be assessed was identified half-way between detection and terminal threshold. The fabric method was used to examine a commercial cigarette and a prototype generating known sidestream smoke amounts.

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Determination of heavy metals in mainstream smoke by ORS-ICP-MS.

The pre-treatment process for the determination of ultra trace elements such as heavy metals is one of major factors affecting the reproducibility as well as the uncertainty of the data. Although the ICP-MS instrument has been reported as a reasonable analytical tool, it has also not been providing clear solutions on these difficulties in the analysis of cigarette smoke.

ICP-MS equipped with a collision/reaction cell (CRC) or octopole reaction system (ORS) makes the reduction of particularly argon-containing polyatomic interferences possible by several orders of magnitude. The CRC is pressurised with a gas or a mixture of gases to reduce or eliminate the interfering polyatomic species. In side cell occurs collisional dissociation and gas phase chemical reaction. For the CRC, an He-H_2 mixture was used for reduction of argon-based polyatomic interferences on chromium, arsenic and selenium. In this study using ORS, it was studied how to remove spectral interference and concentrate mainstream smoke in solvent and how to increase reproducibility applying method of direct analysis.

In this study, we compared three different pre-treatment methods, block digestion, microwave digestion and solvent injection, coupled with ORS-ICP-MS for the quantification of heavy metals in mainstream smoke obtained from 3R4F reference cigarettes, and evaluated those efficiencies in the recovery, repeatability and reproducibility.

In all methods, volatile heavy metals, As, showed the highest CV value, and Cd showed the lowest one. However, the solvent injection among the three methods showed the most stable recovery at 96% and a CV value of the reproducibility at 2.36%. This method also showed advantages in time-consumption and compatibility with ICP-MS system.

The results demonstrate that the solvent injection method can be recommended as a superior pre-treatment procedure able to reduce contaminants and spectral interference as well as loss of the elements concerned.

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The use of magnitude estimation to assess the odour and irritation of sidestream smoke - (Part 1) with the cubicle method.

Sidestream smoke, which is the smoke that comes from the lit end of the cigarette, is an area of active research and development for cigarette designers. The overall objective of this research was to develop a 'cubicle' based methodology for the sensory assessment of sidestream smoke. The volume of the cubicles is approximately 1 m^3. The first stage was to ensure that the cubicles were suitably sealed by monitoring the CO decay rates generated from sidestream smoke. The second stage was to identify the optimum amount of smoke to be assessed by building a dose-response relationship between the amount of sidestream smoke and the intensity of the sensory perception (irritation and odour).

To achieve the second stage, a known reference standard (1-butanol) was used to determine the detection threshold under the conditions of the cubicle method. This approach was carried out by generating aerosols from serial dilutions of 1-butanol. The aerosols were generated using an ultrasonic particle generator (SONAER model 241PG) and their respective particle concentrations were measured using a Condensation Particle Counter (TSI model 3022). The aerosols were assessed to measure psychometric functions for the detection of odour and nose irritation. The detection threshold was obtained at the 50% chance-corrected probability point. Panellists were recruited, selected and trained according to the ISO method 8586-1. Panellists were then trained to score intensity ratings using ratio-scaling (magnitude estimation) and a standardized procedure using 1-butanol with a reference (fixed modulus).

Subsequently, panellists were trained to rate a range of sidestream smoke concentrations (reference cigarette 3R4F). The optimum sidestream smoke concentration to be assessed was identified half-way between detection and terminal threshold. The cubicle method was used to examine a commercial cigarette and a prototype generating known sidestream smoke amounts.

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A Diels-Alder reaction among cigarette mainstream smoke components.

The presence of a product(s) from a Diels-Alder reaction between cigarette mainstream smoke components has been described. Data from carbon-13 nuclear magnetic resonance (^{13}C NMR), gas chromatography-atomic emission detection (GC-AED), and gas chromatography-mass selective detection (GC-MSD) revealed a Diels-Alder reaction product resulting from the reaction of benzoquinone (Q), a dienophile, and 1,3-cyclopentadiene, a diene, to yield tricyclo[6.2.1.$0^{2,7}$] undeca-4,9-diene-3,6-dione, more commonly referred to as cyclopentadienebenzoquinone. The reaction between Q and 1,3-cyclopentadiene was observed to have occurred when fresh mainstream vapor phase smoke (VP) from a 2R4F cigarette, captured in acetone, was subsequently treated with Q. Accompanying the Diels-Alder reaction was an additional reaction of Q to form hydroquinone (HQ). These reactions provide additional information on the complexity of cigarette smoke, particularly as it relates to *in situ* reactions involving Q and HQ.

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A study of the reaction between quinone and 2R4F cigarette smoke condensate.

A study using atomic emission detection (AED) investigations to explore the fate of quinone added into 2R4F cigarette smoke condensate (CSC) has been performed. Both natural isotope quinone and ^{13}C labeled quinone were used in the study. When coupled with a gas chromatographic separation (GC/AED), the AED provided informative new data on ^{13}C isotope enriched products generated following reactions between 2R4F CSC and the quinone. Two ^{13}C containing species were detected by GC/AED. Matching chromatographic separation using gas chromatography/mass selective detection (GC/MSD) allowed for a confident structural assignment of a relatively minor CSC $^{13}C_6$ quinone reaction product as nitrohydroquinone ($^{13}C_6NO_2HQ$). The chemical mechanism accounting for the formation of $^{13}C_6NO_2HQ$ in the CSC was envisioned to be a reaction product between HONO and $^{13}C_6$Quinone ($^{13}C_6Q$) to form $^{13}C_6NO_2Q$, followed by reduction of $^{13}C_6NO_2Q$ to $^{13}C_6NO_2HQ$. The amount of $^{13}C_6NO_2HQ$ accounted for ~6% of the added $^{13}C_6Q$. Identical trends in reaction chemistries were found for experiments with $^{12}C_6Q$. The major reaction product detected upon addition of $^{13}C_6Q$ to the 2R4F CSC sample was $^{13}C_6HQ$. $^{13}C_6HQ$ accounted for, on average, ~47% of the initial $^{13}C_6Q$ concentration. Identical trends in reaction chemistries were found for experiments with $^{12}C_6Q$. No additional ^{13}C containing species were detected. A ^{13}C AED compound independent calibration (CIC) approach under the operating conditions was not possible. This body of work further expands the knowledge regarding possible reactions of quinone and hydroquinone in CSC.

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Identification of polar cuticular components on Oriental tobacco leaves by off-line two-dimensional liquid chromatography - mass spectrometry.

Off-line comprehensive two-dimensional liquid chromatography-mass spectrometry was established for identifying the polar fraction of cuticular components in Oriental tobacco. Normal-phase liquid chromatography was chosen for the fractionation of polar fraction of cuticular leaf extract, and the fractions were concentrated with rotary evaporator. Each of the enriched fractions was analyzed by reversed-phase liquid chromatography-electric spray ionization mass spectrometry (RPLC-ESI/MS). 99 compounds were identified including 45 cembranoids, 15 labdanoids, 20 sucrose esters, 3 glucose esters (or fructose esters) and 16 unknown compounds. Of which, 3 cembranoids and 7 labdanoids may possibly be new diterpenoids. Glucose esters (or fructose esters) are also newly found in *Nicotiana tabacum*.

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The effect of thermal decomposition of banded cigarette paper on ignition strength test results.

It is known that the diffusion constant of the banded areas of the cigarette paper is an important parameter for achieving compliance with regulations regarding the ignition strength of cigarettes.

Nevertheless, in many cases, the diffusion constant does not show a good correlation with ASTM E2187 test results. The hypothesis of the present investigation is that this is because the diffusion constant of the banded areas is measured under standard conditions, that is at 23 °C, while for a good performance in the ignition strength test the band has to inhibit the diffusion of oxygen to the glowing cone at much higher temperatures, when the paper and the band material are already partially decomposed.

To investigate this, three-banded cigarette papers with the band material containing a cellulose derivative, starch and a starch derivative, respectively, were heated to temperatures between 170 °C and 290 °C for times between one minute and two hours. The diffusion constant of the bands was measured afterwards and a mathematical model was derived from the data to describe the thermal degradation of the bands. This model was combined with a mathematical model of a smouldering cigarette for numerical simulation. The influence of the degradation speed on the self-extinguishment was simulated and compared to a set of experimental data obtained from cigarettes produced from the three-banded cigarette papers.

It could be shown that the diffusion constant of the thermally decomposed bands on the cigarette paper is a far better predictor of ASTM E2187 performance than the diffusion constant of the banded areas under standard conditions. The model also explains how the band material influences the test results, and can be used to design papers for FSC cigarettes.

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Analysis of glycolaldehyde in mainstream cigarette smoke.

Glycolaldehyde (GA), also known as hydroxyacetaldehyde, has been identified as a major product in the pyrolysis of cellulose under conditions similar to those operating in a burning cigarette. GA has been identified as a mutagen in laboratory studies. Cigarette paper and tobacco leaf contain substantial amounts of cellulose, which raises the possibility that GA may be present in cigarette smoke. However, there is no information available in the scientific literature concerning GA yields in mainstream cigarette smoke.

In order to understand its possible formation in burning cigarettes, mainstream cigarette smoke was analysed for GA. Use of standard methods for the analysis of carbonyl compounds in cigarette smoke (HPLC-UV and GC/MS techniques) were unsuccessful due to co-elution of another, unknown, compound (HPLC-UV) and thermal instability of GA and its derivatives (GC/MS).

A simple and fast method for analysis of GA in mainstream cigarette smoke was developed which overcame the problem associated with traditional analysis methods. GA in mainstream cigarette smoke was trapped by passing smoke through a solution of 2,4-dinitrophenylhydrazine (DNPH) in acetonitrile to prepare a GA-DNPH derivative. No clean-up step was necessary. Detection of the derivative was achieved by LC-MS/MS using APCI in negative ion mode. The limit of detection was 50 ng/cigarette.

The new method was used to measure the levels of GA in mainstream cigarette smoke from a number of cigarette types.

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Contribution of five nitrogen-containing compounds to TPM mutagenic potencies in *Salmonella typhimurium* TA98.

TPM shows mutagenic activity in *Salmonella* mutagenicity assays under metabolic activation conditions. Nitrogen-containing compounds (NCs) in tobacco leaves are considered to be the precursors of some mutagens included in the TPM. The objective of this study was to clarify the contribution of individual NCs in tobacco leaves to the mutagenic potencies of the TPM.

Protein, a mixture of amino acids, sodium nitrate, ammonium chloride and nicotine, which are the major components of the NCs, were individually pyrolyzed using a pyrolysis apparatus model (100% N_2 atmosphere, 16.7 ml/sec. gas flow rate and 800 °C maximum temperature). The pyrolyzed products were subjected to the mutagenicity assay using strain TA98 with metabolic activation. The results for the protein and the mixture of amino acids showed strong mutagenicity, but the others did not. Based on the relationship between the amount of individual components in several single grade tobacco leaves and the specific activity of each pyrolyzed product, the contribution ratio of the total NCs to the TPM mutagenicity generated from the leaves was calculated to be 20-50%.

To identify the effects of interactions with the other components in the leaves, the following study was conducted. Experimental cigarettes were made of leaves that had been added with a 0.5 and 1.0-fold amount of inherent NCs in Burley and flue-cured, respectively. The cigarettes were smoked under ISO conditions and the TPMs gathered were consequently assayed. Although the pyrolyzed product of ammonium salt alone showed no activity, the TPM activity from the flue-cured that it had been added to increased compared with that of the control cigarette. Moreover, the activity of the flue-cured with added protein showed more than the theoretical increase. It was suggested that the impact of the NCs on the mutagenic potency of the TPM was enhanced by pyrolyzing with other components in the flue-cured leaf.

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Collection and analysis of total particulate matter (TPM) and selected Hoffmann analytes using a cigarette smoking simulator.

A cigarette smoking simulator is described which allows targeted smoke components from different smoking materials to be generated when the production of cigarettes is not viable and the analysis of blend alone would not provide the required information.

Initially tobacco (taking out 2R4F cigarettes and three other tobacco blends) was used to test a single-channel version of the simulator. For this purpose, tobacco was ground to an approximate 2 mm particle size and poured into a quartz tube (8 mm i.d.) containing a small plug of standard cellulose acetate filter at one end. Between 0.5 to 1.0 g of tobacco was added to achieve a consistent pressure drop (*e.g.*, 120 mm H_2O) at a length of *ca*. 60 to 80 mm tobacco rod. The quartz tube was loaded into the simulator system, which was connected to a Borgwaldt A14 syringe driver system. Both systems were controlled by an on-board PC. Through a set of user-selectable software parameters, the simulator was able to generate main smoulder and puff parameters similar to those experienced by a burning cigarette (temperatures, heating rates, puff duration, puff frequency and volume). In addition, the assembly of the quartz tube and a standard 44 mm Cambridge filter pad holder was driven by a variable speed motor towards the heating source, allowing the required amount of tobacco to be heated at desired burn rates. Results obtained for the quantification of TPMs, selected aromatic amines, benzo(a)pyrene, tobacco-specific nitrosamines (TSNAs) and phenols showed that the simulator was able to differentiate the four tobacco types. The existing analytical methods for the targeted analytes were able to be applied without modification.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST18

The analysis of coumarin, ferulic acid and caffeic acid in mainstream smoke of 2R4F cigarettes by LC-MS/MS.

Coumarin, ferulic acid and caffeic acid are naturally occurring constituents of tobacco. During smoking of cigarettes, these semi-volatile compounds are expected to transfer to mainstream smoke. Currently there are no routine analytical methods in the tobacco industry to quantify these compounds in cigarette smoke. This presentation describes the experimental work to develop LC-MS/MS methods to quantify these compounds.

As each compound was expected to be present at trace levels in the mainstream smoke, selective and sensitive analytical methods were needed. Kentucky reference cigarettes 2R4F were machine smoked under ISO smoking conditions (35 ml puff, 2.0 second duration at 60 second puff intervals). In addition to the total particulate matter, the filter tips were analysed post-smoking as these compounds are semi-volatile and their selective filtration by cellulose acetate may occur. Cambridge filter pads and the filter tips were extracted with a methanol:water mix at 1:1 ratio. Aliquots of the extracts were analysed directly by LC-MS/MS for coumarin and caffeic acid. In the case of ferulic acid, the extracts were diluted 100 times prior to the analysis in order to reduce suppression. Negative electrospray ionisation was used for the MS/MS analysis of both caffeic and ferulic acid, whilst positive ion atmospheric pressure chemical ionisation was used in the MS/MS analysis of coumarin. The limits of quantification of the methods were 11 ng/cig for coumarin, 75 ng/cig for ferulic acid, and 0.8 ng/cig for caffeic acid respectively.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT15

European Collaborative Study on Cigarette Smoke Analysis (EUCS).

On the basis of a collaborative study on cigarette smoke analysis performed by the German DIN committee, a general Europe wide collaborative study on a yearly basis has been offered by DIN to all interested parties since 2005. The first **E**uropean **C**ollaborative **S**tudy on Cigarette **S**moke Analysis (EUCS) was well received by all participants. The number of participating labs and smoking machines has been steadily increasing from year to year. In 2008 52 labs with 73 smoking machines participated.

This paper gives an overview of the structure of EUCS: participating labs, smoke measurements (NFDPM, nicotine and CO), test cigarettes, statistical report. Distributions of smoke measurements for the different parameters are discussed per year and over the 4 studies, as well as variability and precision measures like r and R in comparison to other studies.

Based on the EUCS data simulation studies have been performed to give information for questions like "necessary number of laboratories for specific investigations" and those results will be presented.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST19

Proposal for a standardised method for waterpipe smoking.

Over the past two years waterpipe smoking has emerged as a fast growing market segment of tobacco consumption. In Europe the smoking of waterpipes has become increasingly popular among young adults and is often their first contact with smoking. There is a perception among young adults that waterpipe smoking is less harmful as compared to cigarette smoking.

Worldwide a lot of discussions and activities are in progress to measure and categorize waterpipe smoking. Testing however, is being completed in the absence of a standardised testing method and equipment, so any published results should be considered with this in mind.

The topic of this poster is the development of a standardised procedure in combination with the technical difficulties relating to such a standardised smoking method. A description of the proposed procedure, as well as first results of smoke components is presented.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT34

The influence of cross-sectional distribution of charcoal in cigarette filters on adsorption efficiency for volatile organic compounds.

Charcoal is one of the most effective technologies applied to cigarette filters to remove volatile organic compounds (VOCs) from cigarette smoke in large quantities and substantially changes the characteristics of the smoke mainly due to adsorption. And a number of extensive research studies have been conducted to optimize the removal potential of charcoal.

The purpose of this study was to evaluate the influence of cross-sectional charcoal distribution in cigarette filters on the adsorption behavior for VOCs in cigarette smoke. Benzene was adopted for evaluation as a typical compound of VOCs, and an adsorption-model of charcoal filters considering the flow of cigarette smoke in filter-tips was constructed by using a fluid analysis software, FLUENT. The experimental adsorption efficiencies of various types of charcoal filters were compared with efficiencies calculated by the adsorption-model assuming that charcoals were homogeneously distributed in cross-section of filter-tips.

In the case of paper-charcoal-filters (PCF), experimental adsorption efficiency for benzene was approximately equal to calculated ones. This result showed that charcoals were distributed homogeneously in cross-section of PCF. As for acetate-charcoal-filters (ACF), on the other hand, experimental value was about 20% lower than calculated value. This result indicated a heterogeneous distribution of charcoals in cross-section of ACF.

It was found that the cross-sectional distribution of charcoals in the filter-tips has a large impact on the adsorption efficiency for VOCs in cigarette smoke.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT32

Determination of organophosphorous pesticides residues in tobacco by LC-MS/MS.

A quick and simple pre-treatment method was introduced for the analysis of 15 organophosphorus pesticide residues in tobacco. The compounds were quantitatively determined by liquid chromatography-mass/mass spectrometry under the MRM mode and methanol/water was selected as the mobile phase. The 15 analytes presented good linear relations in the concentration range of 0.002-0.2 µg/mL ($r > 0.998$). The LODs and recoveries of 15 compounds were 2.58-11.54 µg/kg and 67.7%-104%, respectively. The RSD was less than 9%. Further, the effects of matrix on the results

were examined to optimize the detection conditions. The method was also applied to the residue analysis of agrochemicals besides organophorus pesticides.

Key words: LC-MS/MS, tobacco, organophosphorus pesticide residue, matrix effect

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Propagation of cigarette smolder burn between puffs.

Variation of cigarette paper affects the burn rate during smoking. The static burn rate observed during the actual smoking process [smolder burn] is lower than the equilibrium static burn rate of a statically burning cigarette [static burn]. In this study, the propagation of cigarette burn after puffing was investigated to get further understanding.

The propagation of cigarette burn can be observed as the movement of the paper char line. An image analysis system with a CCD camera was applied to monitor the movement of the paper char line. The surface temperature of the smolder cigarette was measured with a thermograph system.

We found that the paper char line remains stationary for a certain period after puffing, then, once the paper is ignited, the paper char line resumes moving and burn process proceeds towards static burn. We also found that the difference between smolder burn rate and static burn rate increases with time interval between the stopping of paper char line movement after puffing and the re-starting of char line movement by re-ignition of the cigarette paper. According to the analysis of 4 different types of cigarette paper, the time interval increases as the static burn rate decreases.

The study concludes that the propagation of smolder burn is characterized as an intermittent movement of cigarette paper. Furthermore, it explains that the difference between static burn rate and smolder burn rate depends on the re-ignition interval.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT39

Comprehensive studies on triacylglycerol in leaf tobacco.

There has been great discussion about the relation of triacylglycerols with the aroma and taste of cigarettes. However, few studies were conducted to analyze these compounds in tobacco leaves. The problem seems to lie in the fact that known analytical instruments were unable to show detailed structural information. Recently, however, LC/MS has made much progress and APCI (atmospheric pressure chemical ionization) has enabled the display of structural information. Therefore, our research started from the investigation of the analysis on these compounds using this instrument (LC-APCI/MS).

To this end, the mobile phase depended on the NARP (non-aqueous reversed phase) method, which has recently become renowned as the analytical method of low-polar compounds like triacylglycerol. APCI was adapted to the interface of MS to ionize triacylglycerols forcibly. This attempt resulted in the identification of countless triacylglycerols and enabled us to quantify them.

As a result, there were triacylglycerols in flue-cured leaves including mainly unsaturated fatty acid. The total amount of this compound varied in the countries growing flue-cured leaf tobacco. On the other hand, in Burley leaves, not so many triacylglycerols were found. This result leads us to presume

that triacylglycerol amounts in flue-cured or Burley leaves are related to the degree of leaf maturity, respectively. In this report, we discuss the efficacy of the triacylglycerol analysis with LC-APCI/MS and the results obtained in our research.

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Use of an EpiOral™ human tissue model for testing of substances for oral irritancy *in vitro*.

The EpiOral™ tissue model consists of normal human keratinocytes differentiated to form a multilayered tissue. The phenotype is analogous to human oral epithelium *in vivo* providing the opportunity for *in vitro* irritancy testing on human tissue. This study evaluated the effects of potential snus ingredients as irritants using the MTT tissue viability assay as a surrogate for irritancy.

Conditions used were provided by the MatTek Corporation with modifications. Substances were applied topically at 0.3-5000 µg/ml for both 1 and 20h in duplicate. Positive controls, Triton X-100 (1%) and sodium dodecyl sulphate (SDS, 1%), and the negative control, DMSO (0.5%), were used. MTT conversion to formazan, as a measure of tissue viability, was assessed by measuring OD_{570}. Under our experimental conditions, irritancy was defined as a reduction in tissue viability of ≥25%, relative to negative controls.

The tables below show collated control data and results for the tested compounds:

Control	**Exposure (hours)**	**Number of Experiments**	**Average OD_{570}**	**SD**
0.5% DMSO	1	18	1.739	0.2
	20	17	1.472	0.2
Control	**Exposure (hours)**	**Number of Experiments**	**Average Tissue Viability (%)**	**SD**
1% Triton X-100	1	14	37.1	9.7
	20	15	6.7	1.3
1% SDS	1	16	30.7	5.6
	20	16	7.5	1.4

Test Item	**Maximum Concentration (µg/ml)**	**Average Tissue Viability at Maximum Concentration Relative to Controls (%)**		**Irritant Yes/No**	
		1h	**20h**	**1h**	**20h**
Limonene	5000	97.30	100.0	No	No
Menthol	5000	122.7	121.3	No	No
Geraniol	5000	110.3	119.1	No	No
Sodium Carbonate	5000	101.0	98.80	No	No
Citronellol	5000	118.4	124.2	No	No

The MTT viability assay proved to be a reliable and reproducible method to assess EpiOral™ tissue viability with consistent positive and negative control data. Future work will accumulate data for a range of ingredients to collate a database of tissue cytotoxicites (irritancy) or maximum concentrations without an effect.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST09

Comparison of natural porous and perforated tipping paper: effect on harmful smoke deliveries.

The effect of natural porous tipping paper on reducing harmful smoke was investigated, compared with electric spark discharge preperforated tipping (ESP) and Hauni online laser perforated tipping (online laser perforated). The experiments were based on a 84 mm cigarette with 25 mm filter and 30 mm tipping.

The hole size of the three tipping papers was examined by SEM. The natural porous tipping had the smallest hole ranging from 22.8 µm x 13.43 µm to 9.00 µm x 6.02 µm. The hole of ESP and online laser perforated were uniform. Their mean size was 41.90 µm x 41.31 µm and 189.64 µm x 146.67 µm, respectively.

The tar, nicotine, CO, TSNAs, phenols and HCN deliveries of the prepared cigarettes were determined. At the ventilation rate below 30%, tar, nicotine, CO, TSNAs, phenols and HCN of all the samples decreased with the increase of ventilation rate. But the natural porous tipping was significantly more effective on tar, CO, TSNAs, phenols and HCN reduction than the two other tipping papers. Furthermore, it provided the lowest CO/Tar ratio. In addition, natural porous tipping showed low nicotine reduction, which was lower than ESP, but similar to online laser perforated tipping paper, resulting in the highest Nicotine/Tar ratio.

Key words: natural porous, tipping paper, smoke

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT47

Determination of tobacco-specific *N*-nitrosamines in urine of smokers and non-smokers.

Tobacco-specific *N*-nitrosamines (TSNAs) include 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), *N*-nitrosonornicotine (NNN), *N*-nitrosoanabasine (NAB) and *N*-nitrosoanatabine (NAT). IARC class 1 carcinogens NNK and NNN are thought to be important in tobacco carcinogenesis, whereas NAB is a weak rodent carcinogen and NAT is inactive. Assessment of exposure to TSNA by suitable biomarkers is, therefore of great importance.

We developed, validated and applied an LC-MS/MS method for determining total (free and

conjugated) 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol (NNAL, the major metabolite of NNK), and the parent compounds of NNN, NAB and NAT in human urine. The method comprised enzymatic hydrolysis of the glucuronide, solid phase extractions on a molecular imprinted polymer and on a cation-exchange resin, followed by LC-MS/MS analysis. Method performance data met the validation criteria of the US Food and Drug Administration for bioanalytical methods.

108 24hr urine samples were analyzed from smokers and non-smokers, obtained from a larger clinical correlation study representing a useful range of smoke exposures, and looking at a broad range of biomarker analyses. The range of smoke exposures covered by the correlation study were to be used to test efficacy of this new methodology. TSNA levels in smokers' urine were significantly higher than in non-smokers. Urine concentrations from 25 non-smokers were at or below the limit of quantification for NNN (< 2 pg/ml), NAB (< 5 pg/ml) and NAT (< 2 pg/ml). The mean concentration of NNAL in non-smokers' urine was 10 pg/ml. Average concentrations of NNAL, NNN, NAB and

NAT in smokers' urine samples were 126, 7.4, 47.3 and 210 pg/ml, respectively. In smokers, urinary excretion of TSNA significantly correlated with mouth level exposure, and tended to increase with the ISO tar yield of cigarettes.

In conclusion, the newly developed method is suitable for assessing tobacco use-related exposure to NNK, NNN, NAB and NAT.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT29

Development of new Multi-Residue Methods for determination of agrochemicals in tobacco by using LC-MS/MS and GC-MS.

In 2004, we had reported on the Multi-Residue Methods (MRMs) and Single-Residue Methods (SRMs) combination system for targeting 99 agrochemicals listed in the CORESTA Guide No. 1. The MRMs and SRMs analyze 89 and 10 agrochemicals, respectively. The MRMs used GC-ECD, GC-FPD, GC-MS, HPLC and LC-MS as the analytical instruments. GC-MS, GC-ECD, GC-FPD and HPLC were the analytical instruments for the SRMs. The 2004 system gave us satisfactory results in CORESTA proficiency testing of Sub-Group on Pesticides. However, each procedure of MRMs was relatively complicated.

We started to study new MRMs to develop more efficient systems using LC-MS/MS and GC-MS. After several trials, we succeeded in including six agrochemicals, which were analyzed by the SRMs. They were 2,4-D, dicamba, 2,4,5-T, captan, folpet and dinocap. The one agrochemical, camphechlor, was eliminated from the new MRMs, due to low sensitivity. Therefore, the new MRMs can analyze 94 agrochemicals in CORESTA Guide No. 1.

The recoveries of all these 94 agrochemicals were within the satisfactory range; from 70% to 120%. Each Limit of Quantification (LOQ) of 94 agrochemicals was investigated. LOQs of 75 agrochemicals were lower or the same as those obtained by the MRMs of 2004. The other 19 agrochemicals' LOQs were slightly higher than the former MRMs and/or SRMs. However, LOQs of these 19 agrochemicals were the same or less than the GRLs defined in CORESTA Guide No. 1.

The new MRMs decreased the analytical procedure, and the efficiency of our agrochemical analysis was increased compared to the former MRMs. The new MRMs also decreased the consumption of organic solvents for the extraction process.

The detailed analytical procedure including the extraction process of new MRMs will be presented.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT03

Analysis of protein modifications induced by cigarette smoke.

We will present here work done on identification of protein modifications induced by cigarette smoke, particularly protein oxidation and nitration, using *on-line* nanoLC, ECD hyphenation on a FT-ICR instrument (Fourier Transform - Ion Cyclotron Resonance). Briefly proteins were separated on a nanoLC column packed with C4 wide bore phase, then analyzed on FT-ICR mass spectrometer. Intact proteins were fragmented using ECD (Electron Capture Dissociation). This set-up is very efficient as the analysis can be performed on 10 picomoles of proteins allowing us to work on human plasma.

Model proteins (cytochrome C and α-lactalbumine) were firstly oxidized using hydroxyl and nitroxyl radicals generated in solution. Hydroxyl radicals were produced by various Fenton reagents (Cu^{+} or

Fe^{2+} and hydrogen peroxide) whereas nitroxyl radicals were generated by 3-morpholinosydnonimine salt. The mass accuracy obtained using the FT-ICR device coupled to ECD fragmentation, which is better than the 0.1 mass unit for a protein of a molecular weight of 10,000 Da, showed that methionines were oxidized to sulfoxides and peptide backbone fragmentation occurred. For example ß-scissions were observed as indicated by a loss of acetone from valine and leucine residues. The oxidation was further studied on "peptide scale" using a preliminary protein digestion. The generated peptide mixture was analyzed using separation on a C18 nanocolumn and FT-ICR MS/MS. Nitrosation was similarly studied.

In a second step, cigarette smoke produced by a smoking machine was bubbled with or without a Cambridge filter on the same model proteins. The protein modifications will be compared to those obtained by model reactions described above. The study of proteins from human plasma treated with cigarette smoke condensate is currently under investigation.

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Identification and quantification of free radical content in cigarette smoke using nanoLC nano ESI FT-ICR MS/MS.

Cigarette smoke is a highly complex aerosol of several thousand chemical substances of which free radicals, short-life time molecules, are present in high concentration. In order to identify and quantify them, we have developed a new methodology based on nanoLC MS.

Firstly, we will present the technical parameters developed in the laboratory corresponding to the smoking conditions and mass spectrometry developments. Briefly, the method used to trap free radicals was realized by bubbling cigarette smoke in a solution containing a spin trap at a fixed concentration. For these experiments, we used different scavengers like 4,5,5-trimethylpyrroline-N-oxide (TMPO) and 3-amino-2,2,5,5-tetramethyl-1-pyrrolidinyloxide (3-AP). The smoking conditions were a 2-second puff with a volume of 35 mL each 58 seconds using a LM1 smoking machine (Borgwaldt-KC, Germany). For the identification of adducts formed between the free radicals and the scavenger, we developed a methodology based on highly resolutive and accurate mass spectrometer coupled to nanochromatography. The adduct separation and analysis were performed on a C18 inverse phase nanocolumn. The coupling nanoLC nanoESI-FT-ICR-MS and MS/MS parameters were optimized for the *on-line* analysis. These developments have already allowed identifying and quantifying free radicals like CN° and CH_3° and the analysis of other adducts is currently under investigation. The quantification of free radicals was performed by using the deuterated analogue of the spin trap. The intensity of detected signal was shown to be proportional to the number of cigarettes smoked. This linear correlation allows a precise quantitation of the free radicals. Finally we investigated gas-solid trapping using a methodology described by Jan B. Wooten *et al*. based on the interaction of the cigarette smoke with glass beads coated with a spin trap. The comparison between gas-liquid and gas-solid trapping will be described.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST20

Proteomics study of the influence of free radicals contents in cigarette smoke on culture cells.

The influence of free radicals from cigarette smoke on MCF-7 cell lines has been investigated in our laboratory using proteomics study. The work presented here will expose the comparison between untreated cells and cells incubated with conventional cigarette smoke solution.

In order to highlight the MCF-7 modifications induced by cigarette smoke, 2D DIfferential Gel Electrophoresis (2D-DIGE) is the most appropriate method due to its reproducibility and resolution. Briefly, 2D-DIGE consists in labelling proteins from treated and untreated cells by Cy3 and Cy5 fluorescent reagents. The labeled cells are then mixed and separated on the same gel. The gel analysis is performed using dedicated DeCyder software. The analysis was then performed on a platform designed specifically for use with DIGE, spots were excised using a spot picker, proteins were digested in gel using the trypsin by a spot digester and the peptides were eluted and analyzed by MALDI-TOF mass spectrometry. The proteins were then identified by comparing *in silico* digestion of proteins contained in the databank and the experimental spectra using a search engine; in our case the local version of the Mascot software was used. NanoLC nano-ESI MS/MS experiments were performed on a 9.4 Tesla FT-ICR MS instrument allowing sequencing peptides for identification confirmation.

The results obtained using the exposed strategy revealed that several hundred proteins are differentially expressed and that there are several dozen changes in post-translational modification profiles. The analysis of the most intense differentially expressed proteins shows that all the identified proteins behave to the oxidative stress pathway, for example the proteins HspB1: (Heat-shock protein Beta 1) which is known for its implication in the oxidative stress response related to its "chaperone" activity.

Now, the study of protein post-translational modifications *via* the analysis of generated peptides from different enzymes and chemical treatments is under investigation. Moreover, dose-response relationships will be analyzed.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT28

Analysis of agrochemical residues in tobacco using solid phase microextraction-gas chromatography with different mass spectrometric techniques.

A solid phase microextraction (SPME) method in combination with gas chromatography/mass spectrometric techniques was used for the extraction and quantification of 12 selected agrochemical residues in tobacco. The parameters such as the type of fiber, adsorption/desorption time and the extraction temperature affecting the precision and accuracy of the SPME method were investigated and optimized.

Among three types of fibers, polyacrylate (PA), poly-dimethylsiloxane (PDMS) and polydimethylsiloxane-divinylbenzene (PDMS-DVB), PDMS fiber was selected for the extractions of the agrochemicals. The SPME device was automated and on-line coupled to a gas chromatograph with a mass spectrometer. Mass spectrometry (MS) was used and two different instruments, a quadrupole MS and triple quadrupole MS-MS mode, were compared. The performances of the two GC-MS instruments were comparable in terms of linearity (in the range of 0.01~0.5 μg/mL) and sensitivity (limits of detection were in the low ng/mL range). The triple quadrupole MS-MS instrument gave better precision than quadrupole MS system, but generally the relative standard deviations for replicates were acceptable for both instruments (< 15%). The LODs fully satisfied the requirements of the CORESTA GRL. Recoveries of 12 selected agrochemicals in tobacco yielded more than 80% and reproducibility was found to be better than 10% RSD so that the SPME procedure could be applied to the quantitative analysis of agrochemical residues in tobacco. These results demonstrate that SPME has been shown to be a simple extraction technique, which has a number of

advantages such as solvent free extraction, simplicity and compatibility with the mass spectrometric analytical system.

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Absorption model for the characterization and optimization of the lip-release properties of tipping paper.

Commercial tipping paper is usually coated with nitrocellulose in order to improve its lip-release properties while smoking a cigarette. For tipping paper with printed colors applied during a common rotogravure printing process the printing dyes themselves may contain the required amount of nitrocellulose. A homogeneously applied layer of the lip-release substance is necessary in order to exhibit an outstanding hydrophobic behavior and thus to reduce a direct contact of aqueous liquids on the smoker's lips with the paper substrate.

Despite the standardized application procedure for nitrocellulose, the resulting lip-release efficiency can vary between various tipping types. Investigating the absorption effect of coated tipping paper with contact angle measurements, ink floating tests, and time-of-flight (TOF)-SIMS analysis reveals that the efficiency of the lip-release layer depends primarily on the properties of the base paper used. Besides natural paper porosity and the incorporated size quantity, smoothness and the proportion of filler types are also among these basic parameters.

A mathematical model based on the heat diffusion through a thermally excited polymer material will be presented which describes the observed tipping paper absorption effect by combining the fundamental base paper parameters and the nitrocellulose coating properties. The theoretical approximation allows for the prediction of the expected lip-release ability merely from the specifications of the base paper and opens the potential to control the quality of the generated lip-release coating. A sophisticated application of the depicted model might be the possibility to customize tipping paper for target groups with individual smoking habits.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT12

Formation mechanism of *R*-(+)-nicotine in cigarette smoke.

The enantiomeric composition of nicotine in tobacco and cigarette smoke was analyzed by heart-cutting multidimensional gas chromatography (MDGC). The *R*-(+)-nicotine content of the total nicotine in cigarette smoke ranged from 2.6-3.6%, while essentially no *R*-(+)-nicotine was detectable in tobacco. Concerning the formation mechanism of *R*-(+)-nicotine in cigarette smoke, two hypotheses have been put forward in literatures, but none of them has been verified by experimental data. In order to investigate the mechanism of *R*-(+)-nicotine formation during the smoking process, *S*-(-)-nicotine reagent and tobacco were pyrolyzed by a micro-furnace pyrolyzer, and the chirality of nicotine in pyrolyzate was determined on-line by MDGC. The results showed that, no matter whether nicotine was in the form of a pure chemical or in tobacco specimen, the racemization of *S*-(-)-nicotine was synchronous with pyrolytic decomposition. It supports the hypothesis that the high temperatures involved in the smoking process are the main force for the racemization of nicotine. The MDGC system was composed of a pre-column of DB-1 (30 m x 0.25 mm i.d. x 0.25 µm film thickness) and a chiral column of DB-Cyclodex-B (60 m x 0.25 mm i.d. x 0.25 µm film thickness); FID and NPD were used as monitoring and analytical detectors.

Key words: nicotine, racemization, chirality, pyrolysis, MDGC, tobacco, cigarette smoke

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT19

X-Ray absorption techniques for the determination of oxidation states of trace metal constituents of cigarette smoke and ash.

This work describes the first application of synchrotron-based X-ray absorption spectroscopy techniques to study the oxidation state of trace metals present in mainstream cigarette smoke and cigarette ash, using arsenic as an example. Smoke particulate matter was collected on a Cambridge filter pad either at room temperature or cooled by solid CO_2. Smoke samples were subsequently stored at various temperatures (room temperature or under solid CO_2) and for various times (24 to 48 hr). The X-ray Absorption Near Edge Structure (XANES) results showed that the smoke particulate matter contained a mixture of As(III) and As(V) from the Kentucky 3R4F reference cigarettes, while the cigarette ash contained almost exclusively As(V). The ratio of As(V)/As(III) in the smoke samples varied depending on the collection/storage conditions used: a higher ratio was found for the smoke sample that was quenched *in-situ* by solid CO_2 and kept at solid CO_2 temperatures from smoking through to completion of analysis. A lower ratio of As(V)/As(III) for the smoke collected and stored at room temperature suggests that the smoke particulate matter acted as a reducing agent. Extended X-Ray Absorption Fine Structure (EXAFS) analysis revealed that the neighbouring shell of arsenic contained light backscattering atoms (O, C or N) and modelling of the spectra suggested that these are most probably oxygen. There was no evidence to suggest that the arsenic in smoke was present as sulphides.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT24

LIP in Europe, a challenge for the tobacco industry.

The presentation will first consist of a review of the LIP legislation around the world.

Then the results of a study on the changes in cigarette paper characteristics and in the smoke deliveries before and after the US LIP implementation will be shown.

To do this study, 4 cigarette brands having cigarette papers with coated bands and purchased in the US states having LIP regulation, are compared to the same brands coming from states not requiring LIP regulation.

The band designs, and the change in cigarette paper base porosity will be described.

Comparisons between the smoke deliveries will be shown using ISO and intense smoking regimes.

We will see that the main change in smoke deliveries is an increase in CO.

For Europe, the implementation of a LIP legislation requires that the cigarettes also comply with the 10/1/10 regulation.

Possible ways to achieve this, using cigarette paper features and reconstituted tobacco, will be shown.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST23

Determination of total alkaloids as nicotine in tobacco by continuous flow analyzer using potassium thiocyanate.

ISO 15152:2003 describes analysis of total alkaloids as nicotine (TNA) in tobacco, using continuous flow analyzer. The ISO method is based on measurement of colour of the complex generated *in situ* through reactions involving potassium cyanide, a known toxic chemical with LD 50 of 5 ppm.

The titled method based on potassium thiocyanate (KSCN) has been mentioned in the ISO standard as a possible alternative, however, no details have been given. Potassium thiocyanate has LD 50 of 854 ppm and hence is safer to use, handle and store as compared to potassium cyanide.

In the present method, TNA is determined by reacting aqueous extract of tobacco with sulfanilic acid and cyanogen chloride, generated *in situ* by the reaction between potassium thiocyanate and sodium hypochlorite, to form the colour complex. The developed colour is then measured at 460 nm.

All relevant parameters *i.e.* the concentration of reagents, potassium thiocyanate, sodium hypochlorite, and buffer solution, size of sample tubing and length of reaction coil were optimized to obtain results equivalent with ISO method.

The KSCN method has been validated as per standard validation protocols *i.e.* determination of limit of detection, limit of quantification, recovery, precision, repeatability, reproducibility and ruggedness. Recoveries of 97% were obtained with linear regression coefficient of 0.9999. About 800 samples from different tobacco grades with different nicotine concentrations were analyzed simultaneously using both methods. r and R studies on the samples with Nicotine concentration range 0.5 to 3.3% showed the deviations between the results from the two methods to be ±0.1 units.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT36

Methodologies for the detection and chemical characterization of particles released from cigarette filters.

It has been claimed previously that cellulose acetate fibres and carbon granules can be released from cigarette filters during smoking, thereby presenting a health risk to the smoker. The purpose of this study was to conduct an assessment of cigarette filters containing cellulose acetate and activated carbon granules to gain a greater insight into the likely risks involved. The release of particles from cigarette filters containing activated carbon ("Dalmatian") under simulated smoking conditions is compared to the results of a standard cellulose acetate filter using two different methodologies.

Unlit cigarettes are "sham smoked" in a vertical position on a sampling apparatus according to ISO conditions and released particles are collected on a gold-coated capillary pore membrane filter. The loaded filter is then analysed directly using a Raman microprobe: the individual particles are detected and counted in randomly selected areas of the filter and classified into three different types of particles/fibres based on their length/width ratio. Full characterisation can be achieved by microscopic examination combined with a Raman spectroscopic system that allows the assignment of chemical compositions of organic or polymeric particles.

The alternative methodology uses SEM analysis (Scanning Electron Microscopy) for particle counting and characterisation according to guideline VDI 3492, which is generally used for the measurement of inorganic fibrous particles in indoor air.

Although different experimental approaches for particle detection were used, comparable results were obtained. The particles released from cigarette filters were mainly identified as typical cigarette and filter constituents. The results are discussed and compared to the dust exposure for a corresponding

volume of the usual ambient air. Due to the low number of particles released, high variability was observed between single measurements.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT25

Production of LIP cigarettes using the rod method.

As a result of legal regulations, the percentage of cigarettes with self-extinguishing properties is increasing worldwide. Special materials are used, in particular modified papers for the cigarette's tobacco rod, so that the LIP cigarette (Low Ignition Propensity) goes out automatically in a few minutes if not puffed on. This is primarily designed to reduce the penetration of oxygen through the cigarette paper and so stop the cigarette's tobacco rod from burning independently. However, the changes made to the cigarette paper also have a big impact on the production process of the cigarette itself.

The effects of different cigarette papers on the production process on a cigarette maker are tested and described on a classification basis. The following will be addressed: the generation of dust in the rod formation unit, glue application when the cigarette seam is closed, as well as joining the cigarette paper using splice patches or embossing during bobbin changeover. The quality of the printmark, cut quality, seam seal and the attachment of the filter to the tobacco rod will be tested on the finished cigarette. Based on the test results, the links between the material properties of the paper and its processing properties as well as the effects on the cigarette quality will be discussed.

The aim of these tests is to identify how to produce a high-quality cigarette on the one hand, and to achieve stable and reliable production on the other. In particular, a production speed comparable to that known to date using standard papers should be obtained.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT05

***In vitro* micronucleus assay for cigarette smoke using a whole smoke exposure system.**

Previous studies on the biological assessment of cigarette smoke mainly focused on the total particulate matter (TPM) collected with a Cambridge filter or gas vapor phase (GVP) bubbled through phosphate buffered saline (PBS). However, these extracted fractions of cigarette smoke may not completely reflect the biological effects of the actual aerosol. To research the effects of native cigarette smoke *in vitro*, direct exposure methods have been developed recently. Meanwhile, *in vitro* micronucleus (MN) assays have been reported to evaluate the mutagenicity of cigarette smoke.

The objective of this research was to investigate the MN-inducing activity of whole smoke (WS) and GVP using a whole smoke exposure system, CULTEX®, which allows direct exposure of cultured cells to native cigarette smoke. Smoke was generated according to International Organization for Standardization (ISO; 35 ml puff volume, 2 sec duration, once per minute) or Health Canada Intensive (HCI; 55 ml puff volume, 2 sec duration, once per 30 sec, with complete blocking for filter ventilation) conditions and exposed to Chinese hamster lung cells (CHL/IU) cultured on microporous membranes. Dosages were adjusted according to the amount of smoke introduced into the exposure position. The unit of the dosage was indicated as the percentage of cigarette smoke (% of cig.). Under both ISO and HCI smoking conditions, WS and GVP from K2R4F reference cigarettes showed dose-related micronucleus responses and dosages which indicated that the highest micronucleus frequency in WS exposure was about one half that of GVP. The CULTEX® system provides insights into biological effects caused by native cigarette smoke *in vitro*.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST12

Comparison of *in vitro* micronucleus assay between an in-house procedure and a publicly available procedure.

In the tobacco industry, the *Salmonella* mutagenicity assay, the neutral red cytotoxicity assay and the *in vitro* micronucleus assay (MN) are widely used for *in vitro* test of biological activity. It is recognized that the variability of results among laboratories is greater in the MN than in the other assays. The variability will be due to several factors: mainly the diversity in procedures and the difference in laboratory conditions. However, there is little information regarding these aspects.

In our laboratory we have developed an in-house procedure using CHL/IU cells. In addition, a publicly available procedure, the Health Canada T-503 procedure using CHO cells, was conducted. We tried to compare the two procedures in terms of reproducibility and detective power without S9 metabolic activation.

Flue-cured and Burley, and the reference cigarette 2R4F, were used for the comparison. Since the comparison was examined in the same facility with consistency of environment, the differences in laboratory conditions were eliminated. For the evaluation of the results, we developed the 5% effective concentration value (EC5) as a toxicity index. As a result, reproducibility in EC5 of 2R4F with a coefficient of variation of less than 20% in both procedures was realized. In addition, the order of the MN activity of the sample cigarettes was the same. The MN activity of the Burley cigarette was the lowest, with the 2R4F in the middle and the flue-cured the highest in both procedures. No obvious differences were observed in the stability and detective power.

It is suggested that equivalent MN results could be achieved even with different procedures when the tests are examined in the same laboratory. In addition, the toxicity index (EC5) can be used as a quantitative tool for comparative studies of TPM in the micronucleus assay.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT30

Comparison of different clean-up procedures for the determination of plant protection products in tobacco and tobacco products by LC-MS/MS.

In agricultural practice, Plant Protection Products (PPP) are widely used to protect agricultural products against a wide range of pest and plant diseases. As a result of this application and due to the toxicity and persistence of these compounds, the monitoring of PPPs in plant products is required to meet the national residue regulations in different countries.

In recent years, more and more laboratories have successfully implemented the LC-MS/MS technique for the determination of relevant PPPs. Due to the selectivity and sensitivity of LC-MS/MS systems, residues of numerous PPPs can be detected simultaneously.

The influence of tobacco matrices during ionization is a well-known challenge in LC-MS/MS analysis. Therefore, a large variety of procedures for extraction, clean-up and separation were developed by different industries and contract laboratories for the determination of PPP residues.

The well-known DFG S19 method describes an effective sample preparation for the determination of PPPs in tobacco samples. The extracts for LC-MS/MS analysis are obtained by extraction, gel permeation chromatography (GPC) and dissolving in the LC-eluent. As a fact, the DFG S19 method is relatively time consuming.

The application of a fast and simple multi-residue method can significantly reduce the time for sample preparation: the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) multi-residue method is widely applied for PPP residues monitoring in different matrices of plant origin. However, other procedures might also be applicable.

In this work different sample preparation procedures are assessed for the determination of selected PPPs in tobacco matrices, *e.g.* leaf grades and blends, followed by LC-MS/MS detection. Each analyte is quantified by two specific mass transitions. The results of the different sample preparations are compared and discussed.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT51

Uptake of smoke constituents by UK cigarette smokers - A cross-sectional biomarker study.

The exposure to selected cigarette smoke constituents of a representative group of regular UK smokers smoking factory-made cigarettes was investigated. The smokers comprised three groups, 581 smokers of 8-10 mg tar, 207 smokers of 5-7 mg tar, and 38 smokers smoking 1-4 mg tar products. These volunteer numbers represented the ratio of market share in the UK for cigarettes with the respective tar levels. All volunteers were generally healthy males and females aged ≥ 21 years (mean age 34 years). Smoking status was confirmed on their first (screening) visit to the clinic. 103 confirmed non-smoking subjects (mean age 33 years) served as controls. During the second visit to the clinic all volunteers provided a 24-hour urine sample and a blood sample for biomarker analysis, as well as a diary record of their smoking activities for three complete days before attending the study site. Prior to the start of study, the protocol was reviewed and approved by the appropriate ethics committees.

The following biomarkers (for smoke constituents of the particulate and vapour phase) were analysed:

- Blood: COHb (CO), acetonitrile and serum thiocyanate (HCN).
- Urine: Nicotine + five metabolites (nicotine), total NNAL (NNK), 1-OHP = hydroxypyrene (PAH), SPMA = S-phenylmercapturic acid (benzene), HPMA = 3-hydroxypropyl mercapturic acid (acrolein), MHBMA = monohydroxy-3-butenyl mercapturic acid and DHBMA = 1,2-dihydroxybutyl mercapturic acid (butadiene).

The primary objective of the study was to assess the general exposure to selected smoke constituents in the smoking population. Hence, no adjustment of biomarker data for self-reported daily cigarette consumption was made.

Urinary biomarkers were adjusted for creatinine excretion. All biomarker data were then statistically analysed by ANOVA to investigate possible differences between tar groups and demographics (gender etc.).

Average (range) self-reported daily cigarette consumption was 15 (2-54) for the 8-10 mg tar group, 12 (2-38) for the 5-7 mg tar group and 18 (2-45) for the 1-4 mg tar smokers. For all biomarkers with the possible exception of MHBMA, the levels were clearly distinguishable between smokers and non-smokers. Biomarker levels in smokers were highly variable, but generally greatest at the 8-10 mg tar level, lowest at the 5-7 mg tar level and intermediate at the 1-4 mg tar level, with only a few differences observed between genders.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT10

Ultra-slim cigarette performance with alternative smoking regimes.

The unique performance issues present when ultra-slim cigarettes are tested using standard smoking conditions become more significant when alternative smoking regimes are introduced. Smoke-testing regimes specify average volumetric flow rates through fixed puff volume and puff duration. Yet, important characteristics of cigarette performance, like pressure drop and filtration efficiency, depend fundamentally on the smoke velocity. The small face area of a cigarette with 17 mm circumference results in smoke velocities that are roughly twice those found in cigarettes of conventional circumference. Alternative smoking regimes, as specified by regulatory agencies, generally employ larger puff volumes, shorter puff intervals, and possibly partial or complete blocking of the cigarette's filter ventilation system. Larger puff volumes at constant puff duration will further increase the smoke velocities observed in ultra-slim cigarettes. Even partial blocking of the filter ventilation system will amplify this effect in the portion of the cigarette upstream of the filter vents. With standard smoke testing conditions, the contributions of inertial rather than viscous flow to pressure drop are greater in ultra-slim cigarettes than in cigarettes of conventional circumference. Increasing the smoke velocity through the use of intense smoking regimes enhances this effect in tobacco columns and filter tips. With respect to the performance of cigarette filters under standard smoke testing conditions, the efficiency of ultra-slim filters is lower than that of conventional circumference filters at common filter length and pressure drop. With aggressive smoking regimes, the increases in smoke velocity further diminish the efficiency of ultra-slim filters. While ultra-slim cigarettes are unique, their behavior with alternative smoking regimes can be understood through the application of established principles of cigarette performance.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT01

Market survey of the mutagenicity, cytotoxicity and genotoxicity of smokeless tobacco products sold in Canada.

This investigation was undertaken to characterize the range of toxicological responses for the majority of smokeless tobacco (ST) products sold in Canada. A total of 31 ST products were extracted with DMSO (1:9, w/v), in triplicate. These were tested for mutagenicity using the Ames assay using *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 & TA1537 with S9 activation. In addition, a subset of 12 DMSO extracts was tested for cytotoxicity (neutral red uptake (NRU) assay) and genotoxicity (*in vitro* micronucleus (MN) assay). A subset of 4 ST products was also extracted with (i) dichloromethane (DCM) and (ii) artificial saliva (AS). With one exception, all of the 31 DMSO ST extracts were very similar in their mutagenic activity. The greatest response was observed from strains TA100 and TA102 with slight TA98 activity. The maximum activity (TA100) was 30 revertants/mg extracted ST, which is about 3% of that observed for extracts of 'tar' prepared from KR2R4F and greater than that observed from DCM and AS extracts. Cytotoxicity (IC_{50}) of the 12 DMSO extracts ranged from 380 to 8850 µg ST/mL media in contrast to 54 µg/mL observed for KR2R4F 'tar' extracts. When grouped by product type, the order of cytotoxicity was Gutka>Snuff>Pouch>Plug>Long Cut. For most brands, DCM and AS extractants exhibited little or no cytotoxic activity. There was some evidence for a difference in genotoxicity among the 12 ST products; the genotoxicity of DMSO extracts of Gutka and Snuff (combined) being lower than the combined result for Plug, Pouch and Long Cut (averages 0.282 vs. 0.587% MN/mg ST/mL media). However the maximum response was only 7% of that determined for KR2R4F 'tar' extracts. DMSO, DCM and AS extracts were similar in genotoxic activity with the exception of Gutka where the order was AS>DCM>DMSO.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT45

Classification of mainstream and sidestream smoke condensate by headspace mass spectrometry.

Smoke condensate of various cigarettes has been collected after ISO smoking from conventional cigarette filters (mainstream) and Cambridge filters (mainstream and sidestream). Volatile and semivolatile compounds in the filter samples were analysed by using a headspace autosampler hyphenated to a mass spectrometer (AirSense Analyzer). After optimisation of equilibrium and analysis conditions, mass spectra and time dependent signal intensities were recorded and analysed by multivariate data analysis. With this method, cigarettes can be classified according to their composition of volatile and semivolatile mainstream and sidestream compounds, which can then be further related to the cigarettes' sensoric properties (smell, taste, aroma).

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST10

Proposal for an alternative setup of the ASTM test E 2187-ff, standard test method for measuring the ignition strength of cigarettes.

To prevent fatal fires generated by lit cigarettes dropped onto beds or upholstered furniture, a test method for the propensity of a cigarette to ignite soft furnishings is established in many states of the USA.

The test requires that more than 75% of a batch of 40 cigarettes is self extinguishing before reaching the tipping paper while placed on ten layers of Whatman No. 2 substrate.

The test requires the individual doing the test to asses visually both; if the cigarette has self extinguished and the level of charring caused to the substrate. As with all processes requiring individual assessment, results are subjective to each individual and so inherently more variable than a measured test.

The poster deals with the influence of the layer to the glowing process and gives a proposal for a paperless test. Replacing the paper by an inert and reusable part reduces the variance of the results, the costs and may increase the acceptance of the test.

Finally the results of a comparison test against the existing ASTM method done by several laboratories are displayed for discussion.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST27

A fast shop floor determination of triacetin based on microwave technique.

Determination of triacetin content in filter rods has always been an area of detection and measurement that has lagged behind the other areas of measurement in the manufacture of filter rods. The application of triacetin to the processed acetate tow continues to be a critical point in the process, the measurement of which relies on manual weighing (wet & dry) of filter rods or remote laboratory processing. Both methods have high costs either in wasted materials or in laboratory time and materials.

At the CORESTA meeting 2003 in Freiburg this issue was addressed with a presentation that demonstrated a mathematical correlation between the amount of triacetin and the total mass of the filter rod, versus the microwave values A and B for the same filter rod.

But this correlation failed due to a number of reasons.

In 2008 we will show the culmination of this combined technology with an improvement in the level of correlation achieved by integrating pressure drop and diameter of the filter rod to the algorithm.

The study data is provided from the combination of a standard quality parameter testing station for filter rods with a microwave resonator tuned to the triacetin resonance frequency. The study was completed using 10 very different filter tow items. For each tow item, filter rods at 3 different operation points (PD levels) were produced and each operation point was applied with 5 different levels of triacetin in steps of ~20 mg.

The results of these tests are displayed and discussed in this poster presentation.

The data also shows that the greatest level of accuracy can be achieved with a calibration for a specific filter tow type, but even with a universal calibration, accuracy of this measurement technique challenges those achieved by the other methods.

This means that an at-line virtually instant measurement of triacetin is possible today, and dependent on the levels of accuracy required, instrument configurations can be tailored to the specific application.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT14

Research on particle size distribution and component diversity of cigarette mainstream smoke aerosols.

The objective of this paper is to describe the measurement of the particle size distribution and chemical diversity of aerosols in the mainstream smoke of different cigarette brands. Four samples including two kinds of Chinese cigarettes and two kinds of imported ones were smoked according to a standard regime of 2-second puff duration and a frequency of one puff per minute with a puff volume of 35 ml. After an on-line 200:1 dilution, measurements of particle diameter and number concentration were directly conducted by using electrical low pressure impactor (ELPI) with a measurement range from 30 - 10000 nm. Different smoke aerosols were collected based on their size, and the chemical components of different size of aerosols were analyzed by the GC/MS.

The results showed that, the particle size distribution differed between different cigarette brands. During the smoking process, the concentration of smoke aerosols increased puff by puff. The profile of aerosol size distribution peaked in the range of 0.261~0.381 micron for domestic cigarettes and at 0.028 micron for imported cigarettes, and median particle diameter was 0.23 micron for domestic cigarettes and 0.16 micron for imported cigarettes. The aerosol concentration of the four samples were between 2.2×10^{8}p/cm3~3.8×10^{8}p/cm^{3}, and a similar pattern of particle concentration was observed with a range from 2.2×10^{11}~3.8×10^{11} particles per cigarette. At the same time, the smaller the aerosol size was, the better the person felt while smoking. The contents of the chemical components in aerosols with different sizes differed to a certain extent, neophytadiene and indole were more concentrated compared with nicotine. It was also observed that resident time and coagulation are significant factors when determining particle size, and the suitable dilution ratio should be more than 200:1 and resident time should be less than one second.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT50

A study to estimate and correlate cigarette smoke exposure in smokers in Germany as determined by filter analysis and biomarkers of exposure.

A clinical study conducted in Germany compared two methods of estimating exposure to cigarette smoke. Estimates of mouth level exposure (MLE) of nicotine, NNK, pyrene and acrolein were obtained by chemical analysis of spent cigarette filters ('Filter Analysis'). Simultaneous estimates of smoke constituent uptake were achieved by analysis of urinary biomarkers for nicotine (total nicotine equivalents), NNK (total NNAL), pyrene (1-hydroxypyrene) and acrolein (3-hydroxylpropyl-mercapturic acid (3-HPMA)) plus the nicotine metabolite cotinine in both plasma and saliva. The prime objective of this study was to establish the relationship between the exposure estimates obtained by the two methods.

200 volunteer subjects were recruited into the 19 day study; 50 smokers of each of 1-2 mg, 4-6 mg and 9-10 mg ISO tar yield cigarettes and 50 non-smokers. Smokers underwent two periods of home smoking, each followed by confinement in a clinic. In the clinical setting, smoking was permitted *ad libitum* and spent cigarette filters, cigarette consumption data, 24 hour urine, plasma and saliva samples were collected. Diet was controlled to avoid potential interferences with biomarkers and ongoing health checks conducted.

Significant correlations between mouth level exposure and appropriate biomarker levels for all smoke constituents were observed. The adjusted r values were 0.91 (nicotine), 0.85 (NNK), 0.88 (acrolein) and 0.75 (pyrene).

MLE estimates for nicotine, NNK and pyrene showed a dose response in line with ISO tar yield smoked, with 10mg>4mg>1mg, and for acrolein 10mg>4mg=1mg. The exposure estimates from biomarkers also showed a dose response in line with ISO tar yield smoked with 10mg>4mg>1mg>NS, and for pyrene 10mg=4mg>1mg>NS

In conclusion, in this study, smokers of lower yield products were exposed to lower levels of the smoke constituents as determined by filter analysis and by biomarkers of exposure. The estimates from the two methods were well correlated.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST07

Studies on removing polycyclic aromatic hydrocarbons (PAHs) in cigarette smoke by activated carbon fibers loaded with nano-palladium-copper.

The surface of activated carbon fibers (ACFs) was modified with nano scale Pd, Cu and Pd/Cu by chemical retting method. The modified ACFs were characterized by SEM and XPS. The result showed that, in contrast with an unmodified ACF, the surface of the modified ones was much coarser, with the increase in the proportion of micropore, and reduction in the pore volume. Then, representative ACFs were added in cigarette rod based on SEM and XPS results, and the contents of tar and polycyclic aromatic hydrocarbons (PAHs) in cigarette smoke were analysed. It was found that Pd showed higher efficiency than Cu did, and a moderate interfusion of Cu to Pd-ACF showed a significant effect. Among ACF cigarettes, PAHs were removed with a maximum rate of 34.19%, and the corresponding reduction of tar was 19.08%. Meanwhile, the role of Cu for the efficiency of Pd

was discussed by the fact that the interfusion of Cu brought some influence on the crystallization of Pd, which made the crystal smaller or created crystal defects working as centres of higher catalytic activity. In addition, ACFs had little effect on the aroma and taste of cigarette smoking, and neither Pd nor Cu was detected in the cigarette smoke.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST13

A relative activity of main stream smoke of 2R4F by the fractions on *Salmonella* mutagenicity.

Mutagenicity of cigarette smoke is one of the major concerns in smoking and health issues. Reduction of the components possessing mutagenic activity in cigarette main stream smoke can lead to expected reduced-exposure in smokers. In this study we investigated the relative contribution of smoke constituents of 2R4F to mutagenicity to find clues for effective elimination of the components.

TPM was obtained from 2R4F, and several fractions made by various adsorbants including cation exchanger. The mutagenic activity was assessed using *Salmonella* mutagenicity assay with *S. typhimurium* TA98 strain in the presence of metabolic activation system (S9).

The fractions isolated by cation exchange and reverse phase column showed the relatively high mutagenic activity. These results suggest that hydrophilic-cation exchanger and or other adsorbents possessing similar properties may be used to remove the compounds from mainstream smoke.

In this presentation, the fractionation process from mainstream smoke and the relative mutagenic activity of each fraction will be discussed in detail.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT38

Development of a risk-reducing filter.

Several types of active elements in natural plants were selected based on their anti-oxidative characteristics, and the selected active element was incorporated into the filter directly, by a carrier, or in a form of composite filter after granulation. The added antioxidant was modified to increase its proportion in the filter, and cigarettes were made with such filters. The physical characteristics of the filter, routine chemical components in cigarette smoke and changes of free radicals were tested. From the result of analysis that was conducted using HPLC, the stability of antioxidant was confirmed. By setting a temperature condition in which the acceleration of oxidation reaction is improved (a condition in which oxidation reaction does not occur easily), the stability of the filter with antioxidant has improved. The results showed that the free radicals in smoke were reduced by 30% when the amount of antioxidant added to the plasticizer and composite filter totalled 3.0 mg. The process of filter making did not influence the aspect of the cigarette and its routine chemical analytes. There was no apparent change in the taste of the cigarette. The technology of filter making was simple and convenient, the cost of the new filter was inexpensive.

Key words: natural plants, active elements, antioxidant, free radicals, selectively, filter

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST24

Tobacco-specific nitrosamines content in tobacco, cigarettes (and cigarette smoke).

From a toxicological point of view, tobacco specific nitrosamines (TSNA) represent a risk group of tobacco smoke components and, consequently, will be within the scope of research for many years. Knowing TSNA content in tobacco material is a basic starting point for all technological methods aimed at reducing TSNA content in tobacco.

In this paper, an outline will be given on the tests carried out on different tobacco types from various geographical origins to determine TSNA content [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosonornicotine (NNN), N'-nitrosoanatabine (NAT), N'-nitrosoanabasine (NAB)]. Flue-cured, Burley, and Oriental tobaccos were tested, and also tobacco stem and reconstituted tobacco.

Similarly, TSNAs were determined in tobacco and smoke (TPM) of cigarettes manufactured in Croatia and in some cigarettes from EU markets.

Test results suggest that the content of total TSNA in tobaccos range from 0-8 ppm.

Both flue-cured and Burley tobaccos, and cigarette tobacco blends produced in Croatia were found to contain, on average, up to half the content of TSNA found in tobaccos and cigarettes from other geographical areas.

The TSNA content of cigarettes containing 60% or more Croatian tobacco was also found to be significantly lower in comparison to the TSNA content of cigarettes containing tobacco originating from other geographical regions.

Therefore TSNA content in the cigarette smoke of blended cigarettes made mostly from Croatian tobaccos (60% or more) is lower than in the smoke of cigarettes with a comparable design.

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The effect of different smoking regimes on the performance of different weights and activities of carbon in cigarette filters.

For any adsorption process the efficiency of adsorption usually depends on the activity of the adsorbate and the contact time and concentration of the compounds being adsorbed. In a cigarette filter carbon is exposed to a complex mixture of compounds for relatively short periods of time. For more intense smoking regimes smoke passes through filters at higher velocities (shorter contact times) and generally higher yields of smoke compounds are generated. To investigate the effect this has on the performance of carbon in a filter the activity of carbon filters towards a range of smoke compounds including the major vapour phase aldehydes, ketones and hydrocarbons has been studied. Two smoking regimes have been used: the standard ISO regime and the Canadian intense regime. Data will be presented showing cigarette yields and filter retentions, measured by gas chromatography, for the different smoking regimes for filters containing between 15 to 150 mg of carbon for a range of smoke compounds. Also comparisons are made for two different types of coconut shell carbon, a more standard material with a surface area of 1100 m^2/g and a high activity coconut shell carbon with a surface area of 1600 m^2/g.

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Some factors affecting repeatability in smoking Bidi cigarettes.

Indian traditional cigarettes, known as Bidis, are now becoming available worldwide and the machine smoking of these products is of interest to health professionals and regulators alike.

The repeatability of smoking experiments when applied to Bidis can be poor and is often attributed to the "hand made" nature of these products. The variability with which the product is sealed in a smoking machine, together with the natural variability of manufacture is explored through the use of specialised test equipment establishing the sealing efficiency for various rods.

The influence of the variability of three physical factors, weight, inserted PD and length upon yield is examined through the use of full factorial experiments. The yields of CO and TPM are considered for three popular brands of bidi and comparisons made with machine made conventional cigarettes. Recommendations upon selection of parameters to reduce yield variability of bidis to that achieved from machine made cigarettes are presented.

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High throughput puff-by-puff quantification of smoke analysis.

The analysis of cigarette smoke collected on a smoking machine shows that the composition and yields of smoke constituents vary from puff to puff, and depend on the smoking regime. A programme of work has been carried out to minimise the number of analyses and time required.

Reducing time needed for sampling and analysis resulted in improved laboratory efficiency while using the same set of analytical methods.

Taking into account the chemical properties of volatile and carbonyl compounds in smoke, two new parallel devices have been implemented to achieve high throughput puff-by-puff quantification.

Firstly, an automatic puff-by-puff sampling smoking system, followed by a classical "off-line" Hoffmann determination will be presented.

The application of a specific smoking robot VC10 from Vitrocell® in the ISO vs. Intense Regime comparison of carbonyls puff by puff delivery will be shown.

Special attention was given to the geometric design to provide a good day-to-day variability. The limiting factor was then the HPLC determination.

Secondly, for volatile compounds, an "on-line" gas phase puff by puff quantification was achieved. The aim was to shorten the gas chromatographic cycle time, while maintaining resolution.

A smoking machine coupled to a GC-MS system with Gerstel Modular Accelerated Column Heater was proposed. This Low Thermal Mass column oven was used for fast capillary GC analysis, with a target analysis time under one minute. The resulting improvement was significant: separation of mainstream smoke was achieved in approximately 0.6 minutes. Cool-down and equilibration of the column was achieved in 0.4 minutes.

Comparisons with Tedlar bag and current impinger methodologies were made.

The simplicity of the use of an automated valves system and flexibility for a variety of samples with consecutive run acquisitions will be demonstrated.

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At line measurement of plasticizer content in monoacetate filters using the microwave method.

Plasticizer, commonly triacetin, is a vital addition to cellulose acetate tow to harden cigarette filters and so ensure that they pass cleanly through the assembly process and maintain their designed filter characteristics during smoking. However excess plasticizer causes melt-holes in filters that result in customer complaints and can impact deleteriously on tar yield. Control of plasticizer content is thus crucial but to date there has not been an accurate and effective method of determining plasticizer content in real-time at-line. Wet-dry methodologies are examined and shown to vary greatly and produce inaccuracies in delivery of worse than ±20%. An at-line measurement technique is described that provides quantitative information on both the amount of plasticizer and its distribution within the filter and results of evaluation trials are presented. The accuracy of this method is determined and compared with wet/dry methods. The method is shown to have accuracy comparable with GC methods with results available instantaneously to the user. The use of this method to provide process control information and indication of set-up issues that can result in melt-holes is discussed. The influence of time to measurement on measurement accuracy is explored and recommendations regarding sampling made.

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Systematic study: should diffusivity be considered as a main parameter of cigarette paper?

The property of cigarette paper is usually defined by parameters like porosity, basis weight, chalk content, concentration of burn additive etc. The influences and interactions of these parameters were topics of several studies performed in the past.

In this study a further cigarette paper parameter has been investigated, which was shown to have a significant impact on several key properties and developments of the cigarette industry, especially for CO reduction and LIP. 105 different paper grades have been investigated. The study involved examining the effect of the levels of basis weight, filler content, porosity, burn additive content and type and pulp grade. The statistical evaluation of the results showed significant influences and interactions of several paper parameters on the diffusivity of cigarette paper.

In a second stage the impact of thermal treatment was investigated. The paper samples were treated at elevated temperature for a defined time. It could be shown that relevant effects can be observed only for the heat treated papers.

Finally it could be shown that diffusivity is a fundamental property of cigarette paper for mainstream smoke yields and LIP performance of a cigarette.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST25

Analysis of Amadori compounds in tobacco by high-performance liquid chromatography coupled to tandem mass spectrometry.

Amadori compounds, 1-amino-1-deoxy-2-ketoses, represent a key class of Maillard reaction intermediates. Under different reaction conditions, Amadori compounds generate various aromatic substances, which enhance the smoking quality of cigarette products. Therefore the compounds are important precursors of tobacco aromatic substances. A simple and efficient HPLC-MS/MS method was developed to qualitatively and quantitatively analyze three major Amadori compounds in tobacco. The rule of their tandem mass spectrometry was studied and their contents in different tobacco products were determined.

Key words: Maillard reaction, amadori compounds, tobacco, HPLC-MS/MS

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT37

Study on the application of macro-porous carrier / polyamine composite materials in selective filtration of aldehydes in cigarette mainstream smoke.

The distribution of vapor phase aldehydes in the whole mainstream smoke was demonstrated by HPLC. It indicates that the distribution of aldehydes in vapor phase of smoke was related to aldehyde's chemical reactivity. Formaldehyde, acrolein and crotonaldehyde had higher chemical reactivity and existed in particulate phase at a greater proportion. Some modified porous carriers were screened. Macro-porous silica gel modified by MCA (Melamine derivative), PEI (Polyethyleneimine), Ammonium Polyphosphate with high polymerisation degree, have good filtration performance for formaldehyde, acrolein and crotonaldehyde in smoke. The results showed that amounts of formaldehyde, acrolein and crotonaldehyde released in smoke might be reduced by 30%, 25%, and 15%, respectively.

Surface of macro porous silica gel was modified by A-172 (Vinyl tris-(2-methoxyethoxy)silane), KH570 (γ-methacryloxypropyltrimethoxysilane), coupling agent with carbon-carbon double bond, then macro-porous silica gel/polyamine were prepared, in which surface of carrier was modified by *in situ* polymerization with functional monomer of carbon-carbon double bond. These materials had good selective filtration behavior, the result showed the amount of formaldehyde, acrolein and crotonaldehyde in cigarette smoke release could be reduced by 40%, 20%, 20%, respectively.

Key words: smoke, aldehyde, polyamine

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***In vitro* tests with fresh cigarette smoke - Effects of charcoal filters on whole smoke and vapour phase mutagenicity and genotoxicity.**

In vitro analysis of fresh smoke is an essential part of cigarette testing. The exposure methods employed should be reflecting human smoke exposure. Whole smoke is a dynamic system and has to be handled accordingly. In the space of few seconds smoke changes its properties. Therefore, the period of time until the smoke is in contact with cells has to be kept as short as possible. The same preconditions need to be considered for the vapour phase. For realistic assessment and comparison of biologic effects of whole smoke and its vapour phase the conditions of the tests have to be the same.

Cigarette Smoke Condensates (CSC) tests reflect only part of cigarette smoke's biological effects. In particular, the effects of charcoal filters on vapour phase are only insufficiently detectable with standard *in vitro* methods for cigarette testing (Health Canada methods T 501, T 503).

In this study fresh smoke mutagenicity and genotoxicity of two charcoal cigarettes was compared with a Cellulose Acetate (CA) filter cigarette. The three American Blend cigarettes had similar puff numbers and tar yields.

Fresh Whole Smoke (WS) and Vapour Phase (VP) were tested for mutagenicity and genotoxicity in the Ames test and the *In Vitro* Micronucleus assay (IVM, air-liquid system).

For the detection of mutagenicity with the Ames test our fresh smoke treatment liquid system with *S. typhimurium* TA100 was used. The test showed high response to whole smoke and vapour phase. Charcoal filters decreased the whole smoke mutagenicity in comparison to a standard CA filter up to 46% and the vapour phase mutagenicity up to 61%.

The IVM tests with V-79 showed significant reductions of WS and VP activity compared with CA filter cigarettes.

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Cancer risk calculations for mainstream smoke constituents from selected cigarette brands: concordance between calculated and observed risk.

A quantitative cancer risk assessment of mainstream smoke (MSS) constituents was performed for thirty-five (35) U.S. commercial cigarette brands. Products represented a broad range of "tar" yields (0.9 to 19 mg) as determined by machine smoking using the Federal Trade Commission puffing regime. Cigarettes were machine-smoked using an intense puffing regime (60 ml puff volume every 30 seconds with 2 second duration; 50% vent blocking) and yields of twenty-three (23) individual MSS constituents measured. The constituents studied in the risk assessment were selected on the basis of their detection in MSS, evidence of being known or suspected carcinogens, and availability of cancer potency data. Compounds meeting these criteria fell into a range of chemical classes, including polycyclic aromatic hydrocarbons, semi-volatile organics, heavy metals, tobacco-specific nitrosamines, aromatic amines, and carbonyl compounds. Data incorporated in the point estimate model include 95th percentile daily cigarette consumption from the 1999-2002 National Health and Nutrition Examination Survey (NHANES) as well as exposure duration, averaging time, and lifespan consistent with current cancer risk assessment practices. Cancer risk estimates for the individual MSS constituents ranged from 3.1×10^{-7} (benzo-[k]-fluoranthene) to 2.5×10^{-2} (1,3-butadiene). Summation of cancer risk values calculated from all individual constituents suggested that several compound classes weighed more heavily in the overall calculated cancer risk for the cigarettes in the study. Compared to previously reported models, this approach aligns better with the observed smoking-related cancer incidence in the U.S. population, with the outcome dependent on input assumptions such as smoking regime, exposure scenario, and cancer potency data. The model does not account for variability in cigarette yield, internal dosimetry, or interaction between chemical constituents.

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Development of a risk-based priority toxicant list for smokeless tobacco products.

Quantitative risk analysis (QRA) is an analytical process for quantifying the probability and potential impact of a defined risk. We have developed a priority toxicant list for comparing smokeless tobacco products by applying QRA principles based on cancer risk data rather than epidemiological data to 44 constituents analyzed through surveys of products in the U.S. smokeless tobacco market. Analytes

included the GothiaTek® analytes and additional selected heavy metals, volatile *N*-nitrosamines, tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons (PAHs), polyaromatic amines, volatile carbonyls, other volatiles, and acrylamide. Product categories surveyed included moist snuff, dry snuff, snus, plug, loose leaf, and a dissolvable pellet. Constituents were evaluated based on their International Agency for Research on Cancer classification and for plausible roles in the etiology of smokeless tobacco-related disease. Peer-reviewed oral toxicity data in the form of cancer slope factors (CSFs) were available for 23 analytes. Major assumptions included a daily consumption rate of 16 g (wet weight), complete bioavailability of the constituents studied, and body weight, years of use, and 70-year lifetime consistent with current risk assessment practices. The calculated cancer risk based on maximum yields for 17 constituents exceeded the generally acceptable cancer risk level of 1×10^{-6} with lead presenting the lowest risk (1.5×10^{-6}) and NNK presenting the highest (7.9×10^{-2}). Calculated cancer risk based on GothiaTek® limits exceeded 1×10^{-6} for all analytes for which oral CSFs were available. A ranking based on calculation of cancer risk included heavy metals, PAHs, *N*-nitrosamines, and acrylamide in a priority toxicant list. This risk-based approach has utility in stewardship evaluation of smokeless tobacco products and may serve as a basis for identifying target constituents in future toxicant reduction efforts.

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The effect of cigarette design variables on ISO Hoffmann analyte to tar ratios.

Cigarette design variables such as filter ventilation are capable of lowering all smoke constituent yields to give a lower tar yield product. To selectively remove a Hoffmann analyte the reduction of the Hoffmann analyte should be greater than the overall tar reduction.

An experimental design approach (central composite design) to investigate the effects of filter pressure drop, cigarette paper permeability, filter ventilation and lamina tobacco type on mainstream Hoffmann analyte/NFDPM (tar) ratios under ISO machine-smoking conditions will be presented.

The filter pressure drop range is between 40 to 120 mm water gauge, cigarette paper permeability from 10 to 100 CORESTA units, on-line laser filter ventilation between 0-70% and four lamina tobacco blend styles are Virginia, Burley (uncased), Oriental and a 1:1 mixture of Virginia/Burley.

Different fractions of mainstream smoke can be represented by benzo*(a)*pyrene (particulate phase), pyridine (semi-volatile), acetaldehyde (vapour phase) and carbon monoxide (gas phase). Results from the Virginia blend indicate that benzo*(a)*pyrene/NFDPM ratio remains constant over the filter pressure drop, paper permeability and filter ventilation ranges used. The pyridine/NFDPM ratio decreases with increasing filter ventilation indicating that it is selectively removed by this cigarette design parameter. The acetaldehyde/NFDPM ratio increases with increasing filter pressure drop, which means that the NFDPM is selectively removed in preference to acetaldehyde. Carbon monoxide is selectively removed by increasing the filter ventilation whilst NFDPM is selectively removed in preference to carbon monoxide with increasing filter pressure drop. A more detailed examination of final results will be presented.

This study illustrates how conventional filter and paper design variables can influence the machine yields of Hoffmann analytes in relation to tar when using lamina tobacco blends.

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT48

Simultaneous determination of nicotine and nine nicotine metabolites in urine of smokers using liquid chromatography-tandem mass spectrometry.

A method based on liquid chromatography tandem mass spectrometry (LC-MSMS) was developed for the direct determination of nicotine, cotinine, *trans*-3'-hydroxycotinine, their corresponding glucuronide conjugates, as well as nornicotine, norcotinine, cotinine-N-oxide and nicotine-N'-oxide in the urine of smokers. The assay involves centrifugation and filtration of diluted urine, chromatography was performed on a C18 reversed-phase column using a gradient of 10 mM ammonium acetate, pH 6.8, and methanol as a mobile phase at a flow rate of 1 mL/min. Separated analytes were determined by electrospray ionization tandem mass spectrometry in the positive ion mode of multiple reaction monitoring. Deuterium-labeled nicotine, cotinine, and *trans*-3'-hydroxycotinine were used as internal standards. Calibration curves were linear over the calibration ranges for all the substances under investigation, with $r^2 > 0.998$. Precision (RSD) for all the analytes at high, medium and low levels was between 2.1% and 17%. Recoveries of nicotine and nine of its major metabolites ranged from 78 to 116%. The described LC-MSMS method allows the simultaneous determination of nicotine and nine of its major metabolites in the urine of smokers with good precision and accuracy. Since the method requires a simpler sample pre-treatment and a short time for chromatography (8 min), it is suitable for determining the nicotine dose in mass human biomonitoring studies.

Key words: LC-MS/MS, nicotine, cotinine, *trans*-3'-hydroxycotinine, nornicotine, norcotinine, cotinine-N-oxide, nicotine-N'-oxide

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPTPOST21

Enantiomeric analysis of anatabine, nornicotine and anabasine in tobacco and smoke by MDGC/MS-SIM.

A multi-dimensional gas chromatography and mass spectrometry (MDGC/MS) system with a polysiloxane-based precolumn and cyclodextrin-based analytical column was developed to analyze the enantiomeric compositions of anatabine, nornicotine and anabasine in tobacco and smoke. A simple solvent extraction with dichloromethane followed by derivatisation with trifluoroacetic anhydride gave relative standard deviations of less than 1.50% for the determination of the S-(-)-isomers of all the three alkaloids. The enantiomer abundances of anatabine and nornicotine varied among different tobaccos. S-(-)-anatabine, as a proportion of total anatabine, was 86.64% for flue-cured, 86.02% for Burley and 77.51% for Oriental tobaccos. S-(-)-nornicotine, as a proportion of total nornicotine, was 90.79% in Oriental tobacco and higher than in Burley (69.35%) and flue-cured (58.73%) tobacco. S-(-)-anabasine, as a proportion of total anabasine, was relatively constant for flue-cured (60.07%), Burley (65.07%) and Oriental (61.65%) tobaccos. The enantiomeric compositions of the secondary alkaloids in cigarette filler were in accordance with the tobacco blends. The enantiomeric purities of anatabine and anabasine decreased during smoking process and these results were in conformity with the rule of thermal racemization. The enantiomeric purities of nornicotine increased during smoking contrary to the general rule of thermal racemization.

Key words: MDGC, GC/MS, enantiomeric analysis, nornicotine, anatabine, anabasine, tobacco; tobacco smoke

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Simultaneous determination of six solvent residues in cigarette packaging materials by purge-and-trap connected with tandem dual detector-gas chromatography.

A capillary gas chromatography for the simultaneous determination of solvent residues in cigarette packaging materials was developed by the purge-and-trap sampling technology and tandem PID/FID (Photoionization/Flame Ionization Detector). The conditions of purge-and-trap were optimized by orthogonal experiment. Six solvent residues (ethanol, acetone, ethyl acetate, benzene, 4-methyl-2-pentanone, and toluene) in cigarette packaging materials were qualitatively and quantitatively analyzed under the optimized conditions simultaneously. The detection limits of the method ranged from 0.054 to 0.688 ng. Recoveries and relative standard deviations were 99.6~107% and 0.21~5.74%, respectively. The proposed method shows the advantages of simple pre-treatment, rapid analysis, high precision and sensitivity, which is a powerful tool that can be applied for the determination of solvent residues in cigarette packaging. The volatility tendency of solvent residues in several cigarette packaging materials was further studied from two aspects, namely, storage condition and storage time. The results indicated that the content of solvent residues in cigarette packaging materials under the open condition reduced more quickly than under the closed condition. Furthermore, they reduced evidently in the first seven days, and then reduced slowly. This investigation is very important for monitoring the content of solvent residues in cigarette packaging materials, which contribute to improving the security of tobacco.

Key words: Purge-and-Trap, Tandem Dual detector, gas chromatography, solvent residues, cigarette packaging material

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Fingerprints of cigarette smoke by fast GC: application in quality evaluation.

The evaluation of cigarette smoke plays an important role in cigarette design and maintenance. Although the components of smoke are very complicated, gas chromatography fingerprints is a good method that has been used to objectively evaluate the quality of smoke. A great number of gas chromatographic data are needed in the method in order to make analytical results reliable. Fast GC is a convenient analytical method with high resolution. Abundant stable and repeatable information can be obtained when fast GC is applied to smoke analysis. Moreover, it takes a shorter time to get a great deal of gas chromatography fingerprints of smoke. After comparing the analytical results of numerous components in the trimethylsilylation cigarette smoke condensate by fast GC and common GC, it is found that fast GC only takes 38 minutes to finish the analytical process, however, the latter needs 100 minutes. Furthermore fast GC can provide good separation and repeatable data. Using fast GC to analyze trimethylsilylanized cigarette smoke condensate is an efficient way to evaluate the cigarette smoke via fingerprint. PCA recognition and fingerprint standard method by TAI are used to treat with fast GC data of five kinds of trimethylsilylation cigarette smoke condensates. It is proven that the results can evaluate the quality of smoke quantitatively and accurately.

Key words: fast GC, fingerprint, cigarette smoke, quality evaluation, PCA pattern recognition

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CORESTA Congress, Shanghai, 2008, Smoke Science/Product Technology Groups, abstr. SSPT31

Analysis of 29 organophosphorus pesticide residues in tobacco and their transfer behavior by GC-MS.

A new method for determining 29 organophosphorus pesticide residues is developed. The extraction of the analytes is performed using the accelerated solvent extraction technique, and the extract is concentrated using a Vortex evaporator. Then the solid-phase extraction procedure is applied to the concentrated extract. Finally, the samples are analyzed by gas chromatography-mass spectrometry. The compounds are determined qualitatively by retention time, and determined quantitatively by internal standard, mirex, and the average recoveries ranged from 61.35% to 128.09%, with the relative standard deviations (RSD) below 12%. This method is fast, effective and automated. Cigarettes made of flue-cured tobacco added with different amounts of organophosphorus pesticides are used for testing the behavior of the pesticides transferred into mainstream smoke and retained by the cigarette butt. For all the organophosphorus pesticide residues, n.d.-6.3% are transferred from tobacco into main-stream smoke, and 0.3%-15.0% are retained by the cigarette butt.

Key words: tobacco, transfer rate, accelerated solvent extraction, solid-phase extraction, gas chromatography/mass spectrometry, organophosphorus pesticide

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A novel chemiluminescent immunoassay for evaluation of the protective effect of traditional Chinese medicine on DNA damage induced by cigarette smoke.

Tobacco smoking can produce a large amount of harmful components such as free radicals, reactive oxygen species (ROS) and polyphenols. These compounds can be activated and attack DNA, which results in DNA damages.

This study is to develop a novel chemiluminescent (CL) immunoassay for the evaluation of the protective effect of Traditional Chinese Medicine (TCM) on the DNA damage induced by cigarette smoke (CS). This method takes advantage of a magnetic separation/mixing process and the specific combination of anti-8-OH-dG with 8-OH-dG induced from oxidative DNA damage. It is mainly composed of the following six steps: (1) DNA is immobilized on the surface of magnetic beads, then TCM is added in the solution; (2) different oxidants such as Fenton reagents, CS are selected to oxidaze DNA; (3) After a magnetic separation process, the impurity of complex oxidants and the byproducts are removed and the oxidative DNA are kept; (4) 8-OH-dG produced by oxidative DNA damage can be specifically combined with anti-8-OH-dG; (5) HRP labeled second antibody is added to react with anti-8-OH-dG; (6) HRP substrate is added, and thus the CL signal of 8-OH-dG is detected sensitively. This method can be used to study the genetic toxicity of cigarettes and examine the protective effect of TCM components on oxidative DNA damage induced by CS. The degrees of DNA damage from ROS and CS were studied and the relatively obvious inhibitory effects of TCM components on 8-OH-dG formation were obtained. Various TCM components were screened by this method. The results suggested that several TCM components such as emodin, quercetin, isofraxidin, ginsenoside, astragaloside, gardenoside and naringenin can significantly reduce DNA damage induced by CS. This study provides theoretical support for developing efficient and safe TCM preparations, which can be added to the tobacco rod, to reduce the risk associated with cigarette smoking.

Key words: DNA damage, 8-OH-dG, immunoassay, TCM components, cigarette smoke

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Quantitative analysis of volatile carbonyls in sidestream cigarette smoke by LC/MS/MS.

Volatile carbonyls have been well known as a possible source of carcinogenicity in cigarette smoke. Accurate determination of volatile carbonyls is a great help for assessing the risk of cigarette to public health. We have developed and validated a specific and sensitive method to quantitatively analyze volatile carbonyls in side-stream cigarette smoke. The cigarette smoke was collected using a Cambridge filter connected to an impinger with acidic solution of 2,4-dinitrophenylhydrazine (DNPH). The collected volatile carbonyls were extracted with acetonitrile/water solution and analyzed by reversed-phase liquid chromatography coupled with quadrupole tandem mass spectrometry in negative electro-spray ionization mode. The method achieved excellent reproducibility. The relative standard deviations were generally below 6%. Limit of detection for every carbonyl was less than 2.8 ng/cig. The recoveries and the accuracies were in the range of 87.2% to 104.7% and 90.1% to 103.3%, respectively. This new method had higher selectivity and sensitivity compared to conventional methods, and achieved the separation of DNPH-crotonaldehyde and DNPH-methyl-ethyl-ketone isomers successfully.

Key words: LC/MS/MS, sidestream cigarette smoke, volatile carbonyls, determination

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Attempt at preparing new hierarchical porous MCM-22 adsorbent.

Smokers want to enjoy tobacco products with minimum health risk. Tobacco companies have a duty to develop such products. To achieve this, one possibility is to use specific adsorbents into the tobacco extract solution during papermaking tobacco reconstitution in which those substances considered to be harmful can be selectively removed. In the research outlined above, we aim to prepare improved zeolite selective adsorbents. These adsorbents will have both micropores and mesopores to maximize their selective adsorption of deleterious compounds such as nitrosamines.

Zeolite MCM-22 is a microporous material so that its potential application in adsorption of bulky carcinogenic compounds such as tobacco specific nitrosamines is limited. Therefore it is necessary to modify MCM-22 through tailoring its pore structure and surface state. As expected, creation of mesopores in MCM-22 could speed up the mass transport while introduction of a strong oxidation organic group in the zeolite could oxidize the nitrosamines. After grafting the $-SO_3H$ group on the MCM-22 treated by alkaline, the amount of N-nitrosonornicotine (NNN) in solution could be reduced by over 70%, considerably more than the parent MCM-22 because of the strong interactions occurring between the modified samples and NNN. A better performance was observed in the experiments using tobacco extract solution. The mechanism of $-SO_3H$ group modification on the surface state to degrade NNN are discussed, giving a clue for the further study on the new nitrosamine trap with high efficiency.

Key words: MCM-22, adsorption, nitrosamines, functionalized, propylsulfonic acid group

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Efficient nitrosamines-trap derived from mesoporous zeolite.

Capturing the carcinogenic constituents such as volatile nitrosamines in tobacco smoke is a challenge not only for the tobacco industry to reduce the harm of smoking, but also for the chemist to prepare

novel selective adsorbent, because it is hard to trap the target with trace amounts among hundreds of other abundant components within a very short time. On the basis of study in adsorption of zeolite and mesoporous silica, we tried to create a hierarchical structure in the new nitrosamines trap, modulating the surface morphology of the channel wall to increase the collision probability between adsorbate and adsorbent. Thus, mesoporous HZSM-5 zeolite was fabricated through impregnating the structure-directing agent into the as-synthesized MCM-41 followed by dry-gel conversion to transform amorphous silica to zeolite crystal. The original surfactant in the as-synthesized MCM-41 was used as the necessary mesoporogen to direct the mesopore genesis of zeolite, and the texture of mesoporous ZSM-5 was tailored by adjusting the Si/Al ratio in the MCM-41 source. The resulting samples were characterized by X-ray diffraction, TEM and N_2 adsorption to evaluate their textural property. Two kinds of nitrosamines with different structure were used as probe molecules in instantaneous or static adsorption to survey the function of the resulting composites. As expected, mesoporous zeolite exhibits a good adsorption capacity in laboratory tests, superior to either microporous zeolite or mesoporous silica. In conclusion, mesoporous zeolites can be the candidate for sieving the carcinogenic constituents in tobacco smoke.

Key words: mesoporous zeolite, transformation, volatile nitrosamines, adsorption

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Single photon ionization time-of-flight mass spectrometry: Investigation on photon cross sections of selected Hoffmann compounds and application on tobacco smoke using the Borgwaldt LM2X-Photon-TOFMS.

In the past few years application of modern analytical techniques, such as time-of-flight mass spectrometry (TOF-MS) with soft ionisation methods have revealed many interesting aspects in complex dynamic matrices, such as tobacco smoke. Unlike the well established off-line techniques (GC, HPLC) there is no need for time consuming trapping, solvent extraction or derivatisation as the composition of multi-component mixtures can be analyzed directly within microseconds. The application of single-photon-ionization (SPI) on various working fields within tobacco research was demonstrated within the past few years. The invention of new sources for intense VUV light especially became of interest for photo ionization techniques, such as SPI and provides a useful tool for the construction of rugged instruments, such as the Borgwaldt LM2X-Photon-TOFMS.

In principle, the use of photons proves very useful in terms of quantification as each component inhibits distinctive interactions with photons of specific wavelengths. In a mixture of various different compounds quantification is possible knowing only one exact concentration and the specific relative cross sections of the other compounds to this substance. In this study cross sections of more than 50 compounds with suspected hazards to health were determined for two different wavelengths of VUV-light sources (Ar- and Kr-EBEL) and application was demonstrated on a set of 2R4F research cigarettes using the Borgwaldt LM2X-Photo-TOFMS.

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