

**ABSTRACTS OF PRESENTATIONS MADE AT THE
2005 CORESTA JOINT MEETING OF THE
SMOKE SCIENCE AND PRODUCT TECHNOLOGY STUDY GROUPS
STRATFORD-UPON-AVON, UNITED KINGDOM**

(by alphabetical order of first authors)

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 40

Introduction of a new simple and rapid multi residue method for the determination of agrochemical residues in tobacco by LC-MS/MS.

Multi Residue Methods are playing an important role to monitor pesticide residues in tobacco. In order to reduce costs of analysis and turn-around times the so-called QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) multi residue method [1] was adapted to tobacco. The intention was to establish a supplementary method to the DFG S19 method, developed by Dr. Specht & Partner and very extensively used in our lab for the determination of pesticides in tobacco.

Extracts obtained from the QuEChERS sample preparation are injected into a LC-MS/MS system equipped with electrospray- and atmospheric pressure photo ionization sources (ESI and APPI). The APPI ionization was applied for the determination of quite unpolar, rather GC amendable compounds, like captan and folpet.

The sample work-up procedure consists of the following steps:

- after the addition of water the fine grounded tobacco material is extracted with buffered acetonitrile followed by salting out liquid-liquid partitioning with magnesium sulfate and sodium acetate;
- the organic phase, separated by centrifugation is further cleaned-up by dispersive-SPE with a combination of primary secondary amine adsorbent (PSA) and magnesium sulfate in order to remove organic acids, excess water and other matrix components;
- the final determination is done by LC-(ESI/APPI)MS/MS.

To our knowledge, this is the first attempt to adapt the Quechers method, that initially was developed by Anastassiades *et al* for the determination of pesticides in fresh fruits and vegetables to tobacco. The big advantage of this method is the noticeable reduction of costs and turn-around times due to simplification of the sample preparation procedure.

Up to now, 31 of the 99 pesticides, listed in the CORESTA ACAC guide were tested with satisfactory recovery rates (70 – 110%). Our aim is to present as much as possible recovery rates for those pesticides that are amenable to LC-MS/MS detection at the advised Guidance Residue Levels.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 52

Comparison of menthol migration from filler to filter and mainstream smoke with leaf tobaccos.

The migration of menthol from the filler to filter and mainstream smoke was compared with leaf tobacco types. The leaf tobaccos used were local flue-cured lower leaves (KFL), flue-cured upper leaves (KFU), burley (B), and expanded tobacco (EX). The filter attached to the cigarette was cellulose acetate (2.7/35,000) containing 6% triacetin. The 0.8% of menthol was applied into the fillers via top dressing, respectively. Menthol migration to the filter and mainstream was measured at the constant condition every 15 days for two months by a gas chromatography with DB-WAX column and FID detector.

A big difference in migration of menthol into the filter part was observed between leaf tobacco types as 59% in KFL, 53% in B, 48% in EX and 36% in KFU, respectively. Migration of menthol to the mainstream smoke showed same order with the leaf tobaccos. Namely, the amount delivered to mainstream smoke was 14.7%, 11.8%, 10.8% and 10.2%, respectively.

The results suggest that flue-cured has the highest holding ability against menthol, on the contrary, burley tobacco has relatively a low holding ability among the leaf tobaccos tested. In the present, the holding ability of others including oriental, reconstituted tobacco and Virginia leaf tobaccos against menthol will also be discussed.

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The retention of smoke constituents in the human respiratory tract.

Measurements on the retention of cigarette smoke constituents in the human respiratory tract have been undertaken for more than 100 years. The first studies on nicotine retention were begun by Lehmann in Germany in 1903 and published in 1908. The first studies on the retention of smoke particulate matter were published by Baumbereger in the U.S.A. in 1923. Since those early publications many studies have been undertaken, more or less continuously. This paper will review the work that has been done over the last 100 years, including a large number of unpublished studies undertaken by British American Tobacco in Southampton, U.K. The techniques used have evolved over the years and there is a certain amount of variation in the data. However, the general trends in the results are reassuringly consistent. The bulk of the studies indicate that, on average, 60 to 80% of the mainstream smoke particulate matter is retained in the lungs after inhalation. For nicotine, carbon monoxide, nitric oxide and aldehydes the total retentions are of the order of 90-100, 55-65, 100 and approximately 90% respectively during cigarette smoke inhalation. For most smoke constituents the retentions in the mouth only are considerably smaller than in the whole respiratory tract. The retention values are dependent on the depth of inhalation, hold time in the lungs, exhalation volume and other factors for most smoke constituents. However, the degree of nicotine retention following inhalation is not markedly influenced by changes in respiratory parameters. Furthermore, the percentage retentions are smaller for non-smoking subjects exposed to environmental tobacco smoke. The smoke retentions are related to properties of the smoke aerosol particles and gases and their behaviour as they travel through the respiratory tract.

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The generation of formaldehyde from saccharide tobacco ingredients.

In recent years much effort has been devoted to assessing the influence of tobacco ingredients on the chemistry and toxicity of cigarette mainstream smoke. The techniques used to make the assessments are the pyrolysis of the ingredients and determination of the products of pyrolysis, especially those that are toxic, the influence of the ingredients on smoke constituents believed by regulatory authorities to be relevant to smoking-related diseases, influence of the ingredients on the *in vitro* genotoxicity and cytotoxicity of smoke particulate matter, and influence of the ingredients on the inhalation toxicity of smoke. Progress in the work undertaken by British American Tobacco has been presented at CORESTA meetings held in 2002, 2003 and 2004. All of the studies have indicated that commonly used tobacco ingredients do not change the toxicity of smoke as measured in specified assays. Also, the ingredients have no effect on the levels of most smoke constituents that may be relevant to smoking-related diseases. One possible exception to this generalisation is formaldehyde, which is generated from saccharide tobacco ingredients.

In the present study, several individual saccharides commonly used as tobacco ingredients have been added to cigarettes, the cigarettes have been machine-smoked and the yields of formaldehyde in the resultant mainstream smoke have been compared to those from a control (no ingredient) cigarette. In addition, the formation of formaldehyde has been monitored during the pyrolysis of the saccharides. The results will be reviewed and their implications discussed.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 47

Optimising GC/MS conditions to improve pyrolysis analysis of reconstituted smoking materials containing high levels of humectants.

Pyrolysis-GC/MS systems can provide useful information for tobacco and cigarette ingredient analysis. BAT has been using a mixture of 9% oxygen in nitrogen with a ramped temperature programme (300 - 900 °C at 30 °C/s) to simulate the conditions inside the pyrolysis zone of a cigarette [R.R. Baker and L.J. Bishop, "The pyrolysis of tobacco ingredients", J. Anal. Appl. Pyrolysis, 71 (2004) 223-311]. These conditions have appeared to be useful to evaluate many cigarette ingredients. This paper describes the analysis of reconstituted smoking materials (RSMs) that contain high levels of humectants using the same set of parameters. During pyrolysis, these humectants largely transfer directly to the pyrolysate without decomposition.

A polysiloxane-coated non polar DB-5MS capillary column was first trialled because it is suitable for a wide range of polar and non-polar compounds. However, the humectants dominate the major part of the chromatogram, making the identification of other analytes with co-eluting retention times difficult. A high polarity DB-WAX capillary column was then evaluated. This column was found to deliver significantly improved separation.

To illustrate the difference between the two columns five samples were selected for pyrolysis using both columns (two binders, a tobacco blend, a RSM and cellulose). The DB-WAX column allows more peaks to be positively identified for all five samples.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 01

The effect of cigarette design variables on assays of interest to the Tobacco Industry: - 1) experimental design and some initial findings on Hoffmann analyte yields.

Due to the changing regulatory environment in which the tobacco industry operates, there is a requirement to provide significant quantities of data on the mainstream smoke yields from cigarettes. This can include data on Hoffmann analyte yields, and many other assays. Further papers will deal in more detail with Hoffmann analyte modelling and performance, Genotoxicity, blend chemistry, intense smoking, aerosol characteristics and smoker behaviour using some or all of the products described below.

The objective of this paper is to explain the background to all these studies; involving using both conventional and superslim cigarette construction in terms of physical dimensions. Varying the blend types involved using Virginia, Burley and Oriental tobaccos. The experimental format for conventional dimensioned products involved using filter pressure drop, filter ventilation and paper permeability incorporating a statistically based central composite design. The filter pressure drop range being from 40 to 120 mm water gauge, filter ventilation between 0 and 70%, and paper permeability between 10 and 100 Coresta Units.

Initial data analysis is undertaken via a variety of techniques and illustrates the effect of the major cigarette design parameters on Hoffmann analyte yields both in isolation and combination. The interpretation techniques include simple linear responses, three dimensional plots, wire frame plots and the inclusion of check points within the design.

The procedures involved for dealing with experiments of this size and complexity will be given along with the applicability of these approaches, the limitations on the design, measurement and interpretations will be explained.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 06

The effect of cigarette design variables on assays of interest to the Tobacco Industry: - 6) intense smoking regimes.

The objective of this paper is to describe the effect of selected cigarette variables on the measurement of mainstream smoke components under ISO and intense smoking regimes. The experimental format has been described elsewhere by Case *et al*, using products of conventional dimensions, changing filter ventilation, filter pressure drop and cigarette paper permeability based upon a statistically based central composite design. For the intense smoking work the Virginia blend samples were analysed as a complete set, with the Burley, Oriental and Mix cigarette sets having only their centre point and star points analysed to confirm similar patterns throughout blend styles.

Cigarettes were smoked by industry accepted methods for a variety of Hoffmann analytes with a 35 ml puff of 2 second duration and at 60 second intervals with vents open (ISO smoking). These analyses were then repeated using a 55 ml puff of 2 second duration and at 30 second intervals with vents open (intense smoking). The bandwidths of these values were calculated (intense regime yield divided by the ISO yields) and vary with the various Hoffmann analytes.

Both the intense yields and the ISO yields showed the same directional trends across analytes measured. However, the magnitude of these trends did vary across the experimental space. The effect of the design variables on smoke yields under intense smoking regimes was less than under ISO smoking. In general increased filter ventilation increased the bandwidth of the Hoffmann analytes in question.

These comparisons were only made using the two specific smoking regimes described. Other smoking regimes are used within the industry and may give differing trends when tested.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 15

Study on the direct introduction of solvent condensed mainstream smoke for trace elements analysis by inductively coupled plasma mass spectrometry with an octopole reaction cell.

In conventional element analysis, samples of various types are usually prepared or digested and then introduced as solutions to a plasma by a nebulizer. However sample digestion is a tedious, labor-intensive and time-consuming process, and there is a risk of analyte loss and contamination that may occur during sample preparation. The analysis of the trace elements in smoke is not exceptional. Tobacco smoke is composed of around 4,800 compounds, and there are difficulties in the ultratrace analysis of the metallic elements out of various compounds due to the complicated pretreatment. Diverse analytical methods for the metallic elements in mainstream smoke have been presented by specialized institutes in foreign countries, but the outcomes are not coherent.

This study is conducted through gathering the smoke in Isopropyl alcohol (IPA) solutions and injecting it into ICP-MS with the collision reaction cell in order to minimize the pre-processes for the ultratrace analysis of metallic elements of the smoke. The collision and cell techniques help to eliminate spectral interference occurring in the midst of injecting the solvent and reducing remarkably the poly-atomic interference caused by Ca, P, Na, K, C, S, Ar and Cl. The sample used for this analysis is the reference cigarette 2R4F. The concentrated sample in the pure IPA solutions is diluted with 10 times with ultra-pure water of $18.2 \mu\text{S cm}^{-1}$. Major and minor elements of particulate phase gathered by EP (Electrostatic Precipitator) and of gas phase gathered by impinger are confirmed by the semi-quantitative method respectively. In addition, the elements of Hoffmann's List including As, Se, Cr, Ni, Pb and Cd are quantified. This new analytical method shows some advantages such as saving time for digestion, saving solvent for extraction, improvement of reproducibility. The precision of measurement was excellent with %RSD values typically less than 5% for the majority of elements, despite the complex matrix. The recovery was in the range of 85 – 120%.

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The effect of nitrogenous compounds on the generation of formaldehyde from pyrolysis of sugars.

Several studies have reported that formaldehyde is formed during the pyrolysis of saccharides found as tobacco components or used as tobacco ingredients. In a previous study a thermogravimetric analysis-Fourier transform infrared spectrometer (TGA-FTIR) system was developed to study the formation of formaldehyde and other low molecular weight substances from saccharide materials during pyrolysis under 10% oxygen in nitrogen, from room temperature to 900 °C at heating rates of up to 3 °C/s (Baker, Coburn, Liu and Tetteh, *J. Anal. Appl. Pyrol.*, 2005). This system has been used in the present work to study aspects of the mechanism of formaldehyde formation from various sugars used as tobacco ingredients. Although all sugars generate formaldehyde, the amounts vary with the sugar. It is likely that the presence of nitrogenous impurities in the sugars used as tobacco ingredients can inhibit the generation of formaldehyde, as suggested in other studies (*e.g.* Torikai, Tarora, Yoshida and co-workers, papers presented at CORESTA and TSRC, 2003 and published in *Food and Chemical Toxicology*, 2004, 2005). This mechanism has been investigated in the present study by pyrolysing the sugars in the presence of nitrogenous compounds, including diammonium phosphate and the amino acid *l*-proline. The results obtained will be presented and discussed. Based on this work and that published to date, the following can be concluded about the generation of formaldehyde during pyrolysis:

1. Saccharides such as sugars and cellulose are precursors in the burning cigarette to the generation of formaldehyde in smoke.
2. At temperatures below a certain value, of the order of 500 °C, the precursors decompose to give products other than formaldehyde.
3. Some ammonium compounds present in tobacco react with sugars and so inhibit the generation of formaldehyde from decomposition of the sugars. Ammonia also reacts with formaldehyde to form a complex, further suppressing the yield of formaldehyde.
4. Pre-heating tobacco to the order of 250 °C, as in the smoulder period preceding the puff, decreases the formaldehyde generated in the puff.
5. The presence of oxygen in the pyrolysing atmosphere increases the generation of formaldehyde from cellulose and starch, and reduces its generation from fructose and glucose.

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Gene expression profiles in normal human bronchial epithelial (NHBE) cells following exposure to smoke condensate from cigarettes that burn or primarily heat tobacco.

Cigarette smoke is a complex chemical mixture that includes compounds associated with oxidative stress, inflammation and DNA damage. Our laboratory previously reported that smoke condensate from cigarettes that primarily heat tobacco produce less change in p21, GADD45, COX-2 and IL-8 mRNA levels in NHBE cells than smoke condensate from tobacco-burning cigarettes. The objective of this study was to characterize the effect of smoke condensates from tobacco-heating and tobacco-burning cigarettes on genes involved in NF- κ B signal transduction, a pathway reported to mediate in part the response of bronchial epithelial cells to cigarette smoke. In addition to COX-2, genes coding for HO-1, G-CSF, HSP-70 and TNF- α have been identified as being involved in NF- κ B signal transduction. Cigarette smoke condensate (CSC) was prepared from Kentucky 1R4F cigarettes, a tobacco-burning product designed to represent the average low “tar” cigarette in the US market and Eclipse, a cigarette that primarily heats tobacco. Following CSC exposures, changes in mRNA and protein were determined by quantitative RT/PCR and cytokine analyses, respectively. HO-1 and HSP-70 mRNA levels were significantly increased ($p < 0.05$) in cells exposed to K1R4F compared to Eclipse. K1R4F smoke condensate caused a dose-dependent, statistically-significant decrease in TNF α mRNA levels while Eclipse samples were only moderately changed from control values. Cytokine secretion analysis did not reveal differences in TNF- α levels secreted in the media from either condensate exposure, while G-CSF secretion was altered to a lesser degree in NHBE cells treated with Eclipse condensate compared to K1R4F condensate. The present data suggest that the characterization of genes involved in

key signal transduction pathways (i.e. NF- κ B) following cigarette smoke exposure in an *in vitro* human bronchial epithelial cell model may serve as biomarkers of effect for assessing potential reduced-risk cigarettes.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 02

Wavelet transform: improve the transferabilities of NIR calibrations.

Calibration transfer is a difficult task in the application of NIR models. This study introduced wavelet transform algorithm to improve the transferability of NIR calibrations among 5 FT-NIR instruments. The NIR spectra of a group of 400 tobacco samples were used for calibration and validation. Calibrations built with the reconstructed spectra derived from discrete wavelet transform (DWT) on the prediction of tobacco total sugar, reducing sugar, alkaloid and chlorine were studied. Wavelet transform resulted in better transfer performance and lower RMSEV for all 4 constituents. The influence of different wavelet functions and DWT levels on the performance of calibration transfer was also studied to discover the preferable DWT parameters. With the abilities of nonlinear baseline correction and noise filtering, wavelet transform provides promising assistance for the transfer performance of NIR calibrations.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 12

Using paper diffusion measurements to assess the ignition strength of cigarettes.

At previous CORESTA meetings, Schweitzer-Mauduit introduced a simple, graphical method for describing the ASTM ignition strength of cigarettes when the desired ignition performance was achieved through the use of low permeability bands of aqueous film former applied to the paper.

In past work, the variable of choice for predicting cigarette ignition strength has been the paper permeability in the band. However, it has long been known that the diffusion conductance of oxygen to the coal during smolder is the dominant mechanism in providing lower ignition strength in the band and not necessarily the band permeability

In this talk, we will describe a means of measuring the diffusion conductance of banded cigarette paper. We will present data showing that the ignition strength performance of all papers can be normalized using this diffusion conductance measurement. Further, we will show that for a given paper and band construction, permeability can be used as a reasonable measure of diffusion conductance and hence cigarette ignition strength.

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Laboratory proficiency testing for pesticide residue analysis in tobacco.

There is a continuing need for laboratories to demonstrate performance and reliability in pesticide residue analysis. Proficiency testing schemes provide an independent and unbiased assessment of performance, which can be used by participants to evaluate the quality of their analytical procedures. Recently, proficiency testing has become available for laboratories conducting pesticide residue analysis in tobacco through the work of the CORESTA Sub-group on Pesticide Residues under the guidance of FAPAS[®].

The Food Analysis Performance Assessment Scheme (FAPAS[®]) tests the proficiency of food, water and plant health laboratories. Since 1990, FAPAS[®] has organised more than 120 proficiency tests for pesticide residue analysis for participants in 78 countries. FAPAS[®] has carried out assessments from

about 6000 test materials for its three pesticide programs, namely organochlorine pesticides in oils and fats, organophosphorous pesticides in cereals and pesticides in fruit, vegetables, tea and wine. At the request of the CORESTA Sub-group on Pesticide Residues, FAPAS[®] has recently extended its proficiency testing programme to include pesticide residues in tobacco.

Based on the experience gained in the first proficiency trial, done informally by the Sub-group in 2004, a tobacco sample was spiked with 12 pesticides (not known to the participating laboratories) to produce a suitable substrate for the second trial, this time in complete compliance with FAPAS[®]. After the homogeneous distribution of the residues throughout the tobacco had been demonstrated, participants were asked to screen the test material for a range of pesticides outlined by FAPAS[®] (136 pesticide residues in various chemical classes; namely organophosphorous, organochlorine, heterocyclic and nitro compounds as well as pyrethroids, carbamates and amides). The returned data were examined statistically and the proficiency of the laboratories was assessed.

The results of the new proficiency trial (the second done by the Sub-group and the first coordinated by FAPAS[®]) will be presented and discussed together with any conclusions. In particular, the improvements will be pointed out made recently by many participating laboratories, demonstrating that the use of proficiency testing in pesticide residue analysis is clearly paying off for the tobacco Industry.

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The influence of the pore size distribution of cigarette paper on its diffusion constant and air permeability.

It is well known from experimental evidence and theoretical considerations that the diffusion constant of cigarette paper is approximately proportional to the square root of the air permeability. Despite this fact one frequently sees the need to control these two parameters independently, *i.e.* to increase or reduce diffusion without affecting the overall dilution by rod ventilation.

It is, therefore, the goal of this study to investigate to what extent the diffusion constant and the air permeability of cigarette paper can be independently chosen.

To this end a geometrical model of the paper structure is developed. The model is based on the composition, density and pore size distribution of a typical naturally porous cigarette paper. For a single tortuous pore in the cigarette paper the diffusive flow and the viscous flow are estimated numerically and from physical considerations for various pore diameters. By integrating over the pore size distribution the air permeability and the diffusion constant of the cigarette paper are calculated.

As the pore diameter is in good approximation log-normally distributed the effect of variations in mean value and variance of this distribution on the diffusion constant and the air permeability are studied. The results indicate that the possibilities of choosing the diffusion constant D and the air permeability Z independently are limited, because changing the mean value or the variance of the pore size distribution does not cause a significant deviation from the relation $D \sim Z^{1/2}$. A detailed investigation also reveals that cigarette papers with higher permeability show a weaker correlation between the diffusion constant and the air permeability than papers of low permeability. Furthermore the model explains why the coefficient of variation is in general lower for diffusion constants than for air permeability values.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. IG 01

A new system for measuring the toxicity of mainstream cigarette smoke.

Cigarette smoke is a complex mixture of thousands of chemicals distributed between the particulate or 'tar' phase and the vapour phase, which constitute 5% and 95% of whole smoke by weight, respectively. Historically, the *in vitro* toxicological assessment of smoke has only measured the activity

of the particulate phase. However, the vapour phase contains many components, such as aldehydes and oxides of nitrogen, which may induce biological effects. Therefore, we have designed a novel system for exposing *in vitro* cultures of human lung epithelial cells to mainstream cigarette smoke. The cells are housed in a Perspex chamber and maintained at an air-liquid interface. Freshly generated whole smoke is diluted with air and delivered to the chamber to enable direct exposure of the cells. With this technique we have measured the effects of serial dilutions of smoke on cell viability, DNA damage, gene expression and cytokine production using two different *in vitro* cell models: NCI-H292 lung carcinoma cells and differentiated mucociliary cultures of primary lung epithelial cells. Whole smoke reduced cell viability and induced oxidative DNA damage in a dose-dependant manner. Smoke also stimulated the gene expression and/or production of a number of mediators, such as interleukin-8 and matrix-metalloprotease-1, which are implicated in the pathological processes that occur in smoking-related lung disease. We propose that this system may be adapted for studying other aerosols and could help to reduce the use of animals in inhalation toxicity studies in the future by providing an alternative *in vitro* method for evaluating the effects of aerosols and gaseous mixtures.

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Toxicological evaluation of additives for tobacco products – a guide.

Currently no international, European or national Standards in the field of toxicology of additives in tobacco products exist.

The Directive 2001/37/EC of the European Parliament concerning the regulation of tobacco products asks for a statement of additives in tobacco products and its toxicological evaluation. The national implementation of this Directive in Germany (the Tobacco product regulation) was the reason for the elaboration of a guide by the DIN working group “Toxicology of additives”.

The base material for toxicological methods has been compiled and it was evaluated under which basic conditions already existing methods are transferable to the field of tobacco. Not only the toxicological analysis itself but also the subject matter of the preparation and treatment of samples prior to the actual test for ingredients has to be considered.

This guide “DIN Technical Report 133 – Toxicological Evaluation of Additives for Tobacco Products – A Guide” is regarded as a basis for the elaboration of future technical regulations in the form of standards. And it is of importance for the industry as well as for the national inspection bodies in the federal states because it serves as an aid for the preparation and evaluation of toxicological data for additives in tobacco products.

This poster gives an overview of the present state of the art in this field and the first attempts for a test strategy described in the guide.

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The effect of papermaking pigments on carbon monoxide deliveries of cigarettes.

Carbon monoxide deliveries of cigarettes depend on many different factors, including the physical structure and chemical composition of cigarette paper, which in turn is affected by the amount of the pigment in paper, its morphology and chemical composition.

Pigments are used in cigarette papers to improve visual appearance, opacity, color, porosity, ash appearance, etc. These inorganic pigments also enhance the porous three-dimensional structure of cigarette paper, which affects the transport of gaseous molecules such as oxygen and carbon monoxide, thereby influencing cigarette burn rate and deliveries.

The amount of pigment in cigarette paper and its size and shape have a strong influence on the transport of carbon monoxide molecules between the combustion zone and the surroundings, that is, through the

cigarette paper structure. Generally, using smaller pigments increases the overall rate of diffusion of carbon monoxide through cigarette paper.

The chemical composition of the inorganic pigments can also impact carbon monoxide deliveries. Certain pigments, such as some iron oxides, enhance the conversion of carbon monoxide to carbon dioxide and therefore yield lower carbon monoxide deliveries relative to calcium carbonate, which is the dominant pigment used in cigarette papers. There are other pigments, such as certain clays, which yield higher carbon monoxide deliveries compared to calcium carbonates.

This paper examines how the physical properties, amount of pigment, and its chemical composition affect carbon monoxide deliveries from mechanistic viewpoints, which are supported by experimental data.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 53

Analysis of the gas phase of cigarette smoke by Gas Chromatography with UV-Diode Array detection.

A gas chromatography method, coupled with diode–array photometric spectral detection in the ultraviolet region (170–330 nm), was developed for the analysis of the gas phase of cigarette smoke. Several column configurations and elution profiles were employed in an effort to optimize peak resolution and compound identification efficiency. The final optimized method enabled us both to identify and quantify, the presence and the concentration of at least 15 volatiles in the gas phase stream of cigarette smoke. In that way, all major volatile organic compounds (including aldehydes, conjugated dienes, ketones, furans and single ring aromatics), as well as nitric oxide, were analyzed in a straightforward manner through a single chromatographic run, of less than 50 min duration. The method can easily be implemented, by the introduction into the GC injection loop of a small volume from the gas phase steam, directly through the smoking apparatus exhaust circuit, thus providing an excellent alternative to existing available methods, that usually require several extraction steps prior to any chromatographic analysis. Furthermore, all problems concerning ageing of the gas phase are alleviated. The method was additionally proved very reliable as far as accuracy and reproducibility of the results are concerned. Finally, the proposed methodology was implemented through various experimental sets, in order to determine the relative abundance of the various cigarette smoke gas phase constituents in individual puffs as well as in different smoking regimes.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 37

Effect of smoking on the volatile sulfur compounds in oral cavity.

The content of volatile sulfur compounds (VSCs) in oral cavity of 812 volunteers, including 594 smokers and 218 non-smokers, were measured by HALIMETER. The changes of VSCs content in smokers' oral cavities when they sucked different kinds of cigarette and different amount of cigarette were investigated. The results obtained were as follows: 1. The average content of VSCs in oral cavities of smokers is much higher than that of non-smokers. 2. The VSCs content in oral cavity of smokers was correlative with the amount of cigarette they took, especially they took repeatedly. 3. The increase of VSCs in oral cavity of volunteers who smoked *Dongfanghong* cigarette was significantly lower than that of those who smoked control cigarette.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 46

Effects of glycerin as applied to cut tobacco on the variability of tar and nicotine yields in cigarette smoke (III) - the relation between the adhesion force and the surface roughness of cut tobacco.

As previously reported, the variability of tar and nicotine yields (TN yields) in smoke increases with the amount of glycerin applied onto the surface of cut tobacco during the cigarette manufacturing process. This effect was attributed to the adhesion force, due to which the amount of glycerin assimilated by cut tobacco varies as a function of the surface and leads to fluctuations of the filling density in tobacco columns. This in turn leads to fluctuations of the free burning rate (mm/min) and of the burning conditions during the smoking process. Such findings were reported at the CORESTA Smoke & Technology Joint Meeting in 2003 and at the CORESTA Congress in 2004.

We have recently identified a means by which to reduce the amount of glycerin assimilated by cut tobacco. In the study, we examined several kinds of cut tobacco samples. When we observed particularly rugged cut tobacco, we found that over time glycerin accumulates only in the depressions of the surface. The roughness of cut tobacco surface was scanned using a laser microscope. We therefore concluded that the roughness of cut tobacco surface is a key factor in effectively reducing the adhesion force and eliminating glycerin from the contact points between cut tobacco layers.

In this final report, we clarify the relation between the adhesion force and the roughness of the cut tobacco surface, based upon the amount of glycerin retained over time. The above findings will be useful in reducing the variability of TN yields.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 50

PTRMS analysis of tobacco coupled with Principal Component Analysis (PCA) - a novel method for the evaluation of flavour.

Proton Transfer Reaction Mass Spectrometry (PTRMS) is a recent technique used in the analysis of volatile organic compounds. Previously, work has been done in the flavour analysis of cheese, fruits and coffee using PTRMS. Volatile compounds present in tobacco are reported to have a significant correlation with the smoking quality. The possibility of applying PTRMS data obtained by a non-destructive method to evaluate the flavour of flue-cured tobacco from different growth zones has been studied. Six tobacco samples ranked for their flavour character by a human sensory panel were used in the study. The Vial Method of sampling was used to introduce the headspace vapours into a high sensitive PTRMS instrument. No pre-treatment of the milled tobacco sample was done. A scrubbed and humidified stream of ambient air (70 cc per minute) was used to sweep the headspace vapours of the thermostated vial (45 °C) containing 0.5 g of sample into the PTRMS continuously (5 scans). The PTRMS scan range was set from 50-300 amu with a scan rate of 250 ms/amu. Measurable responses were obtained for 200 masses while 40 masses showed statistically significant difference between the six samples. The average of three scans was taken and all samples were analysed in triplicate and the coefficient of variation ranged from 15 to 25%. The average of the triplicate value was used for PCA analysis after normalization of the data. From a two dimensions principal component analysis map, it was observed that the PTRMS was able to discriminate between the six samples. This novel technique can be used for rapid and non-destructive evaluation of volatile compounds responsible for flavour in tobacco.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 34

Factors affecting filter tip colour as a measure of mouth level exposure to tobacco smoke constituents.

The purpose of this investigation was to evaluate the potential effects of puffing regimen, storage time and temperature on filter tip colour and yields in relation to yields of tar, nicotine and CO. Ten brands of Canadian cigarettes, 2 with taped filters, were smoked using a full 3⁴ factorial design (81 smoking regimens; puff volume, 25, 50, 75 mL; puff duration, 1.5, 1.5, 3.5 s; puff interval, 30, 60, 90 s; and butt length filter+overwrap+ 3, 6, or 9 mm). Yields of tar, nicotine, CO were determined and filter colour measurements made immediately after smoking and after 24, and 168 hours storage at room temperature or –10 °C. Filter colour measurements (CIE L*, a* and b*) were obtained from the end of the filter closest to the tobacco column (rod end) using a Macbeth Color-Eye 2020+ with a 4 mm diameter aperture and D65 (average north sky daylight) illumination. Averaged values for L* and a* values were found to be logarithmically related to the tar yield but the relationship with b* was linear. There were highly significant changes in values for L* a* and b* with time when filters were kept at 20 °C which were dramatically reduced by freezing. After 1 week at –10 °C, only a slight increase in yellowness and decrease in redness was noted. With respect to tar yields, the effect of changes in puff interval and puff duration were virtually identical but less than that of puff volume. Moving from a 90 s interval to a 30 s interval or decreasing the puff duration from 3.5 seconds to 1.5 seconds both resulted in an increase in tar yield of approximately 5 mg/cig. Butt length was the least significant factor. Decreasing the butt length from 9 mm to 3 mm increased tar yield by about 3 mg/cig.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 04

The effect of cigarette design variables on assays of interest to the Tobacco Industry: - 4) *in vitro* genotoxic activity of mainstream cigarette smoke.

The modification of cigarette design parameters in a statistically based central composite experimental design framework incorporating paper permeability, filter pressure drop, filter ventilation and tobacco blend and the resulting effects in terms of chemical composition and some aspects of smoker behaviour have been described elsewhere by Case *et al.* The effect of varying these design parameters and the ability of the resultant mainstream smoke to potentially influence ‘in-vitro’ biological activity has also been evaluated using an Ames mutagenicity test and the *In Vitro* Micronucleus test (IVMNT). Both these tests are designed to complement each other in the detection of mutagenic agents and form part of a test strategy as set out in international guidelines.

Cigarettes were smoked under ISO conditions to generate mainstream smoke condensate. Specifically, the genotoxic potential of smoke condensate was assessed by measuring the number of induced revertants in Ames strains TA98 and TA100 in the presence of an S9 metabolic activating fraction. To give an indication of direct acting mutagenic species in mammalian cells the IVMNT with a V79 cell line was used in the absence of an S9 metabolic activating fraction. Following treatment with condensate, cytokinesis blocked cells (Cytochalasin B) were scored for cytotoxicity, as measured by the proportion of binucleate cells and for micronucleus induction within the dividing cell (binucleate) population.

Trends in the data suggest that modifying blend, ventilation, pressure drop and paper permeability in cigarettes design can have an impact on Ames specific activity of the smoke condensate. However the IVMNT results imply that there is little or no change in the specific activity of condensates if design parameters are altered within the limits of the experimental design.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 16

Comparison of the characteristics of low sidestream cigarette papers.

This study was conducted to evaluate the effect of additives in low sidestream cigarette papers, such as $Mg(OH)_2$, TiO_2 and KH_2PO_4 , on the delivery of mainstream and sidestream smoke.

From the analysis of tar and nicotine in sidestream and mainstream smoke, the delivered ratios of tar and nicotine by sidestream to mainstream smoke in common cigarette paper were 5.32 and 8.60, respectively. However, the delivered ratios of those of the paper containing $Mg(OH)_2$ were 2.25~3.23, 4.86~7.14, $Mg(OH)_2$ and KH_2PO_4 were 2.12~2.92, 4.67~6.89, TiO_2 , was 3.21, 7.51 respectively.

The deliver patterns of semi-volatile components in the cigarette mainstream smoke were similar each there, but a slight difference pattern in the amount was observed depending on the kinds of compounds added in cigarette papers. In the cigarettes made of $Mg(OH)_2$ added paper, the aromatic components such as benzene, toluene and phenol were delivered more while the aliphatic components like neophytadiene, ethyl decanoate were delivered less relatively. However, the cigarettes with paper added $Mg(OH)_2$ and KH_2PO_4 showed an opposite trend. The cigarettes made of TiO_2 added paper showed low delivery in the most compounds measured.

In sensory evaluation, cigarette papers tested was noticed a distinguishable sensory character between the low sidestream smoke with additive cigarette papers except TiO_2 added one. Moreover, aroma patterns detected by a electronic nose system in TPM was a similar tendency.

In the present, chemico-sensory properties of smoke from cigarettes made of LSS papers tested will be discussed

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 16

Synthesis and characteristic of the ion exchanger for selective removal of aldehyde compounds in mainstream smoke.

Many analytical chemists have been interested in the aliphatic aldehyde compounds in cigarette mainstream smoke for a long time. To use the filter material for selective reduction of aldehyde compounds in cigarette mainstream smoke, ion exchangers were synthesized by polymerization and functionalized by sulfonation and amination, respectively. FT-IR/ATR was used to characterize functionalized copolymer formation by sulfonation and amination, and the morphology change of the ion exchangers according to the adsorption of cigarette mainstream smoke were observed by SEM. 2R4F Kentucky reference cigarettes were used to investigate selective adsorption characteristics of aldehyde compounds in mainstream smoke. To derive the optimum adsorption condition, we changed the adsorbent type and water contents. The adsorption amount of aldehyde compounds was higher using the anion exchanger than using the cation exchanger in dry conditions. The different adsorption capability was caused by electron delocalization in the carbonyl group. The adsorption efficiency was increased in the presence of moisture. This result indicates that the ion exchanger is available to use as a cigarette filter material because of the large ion exchange capacity and rapid ion exchange reaction.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 05

Microwave drying characteristics of cut tobacco.

The microwave drying characteristics of cut tobacco were studied in this paper. The changes of moisture content and temperature with microwave treatment time were investigated. The microwave drying curve was obtained and the effects of different processing conditions, such as initial moisture content of cut tobacco, power of microwave and vacuum on the drying curve were studied. Based on these, the mathematical model of microwave drying was established. The conclusions showed that: microwave drying of cut tobacco was divided into three phases: increasing-speed, constant-speed and

decreasing-speed. The higher the power of microwave, the more rapid the dehydration was and the more rapid the temperature increased. The higher the initial moisture content, the more rapid the dehydration was. The lower the initial moisture content, the higher the final temperature was. The effects of vacuum on drying characteristic were obscure. The Page equation was found quite suitable to describe the dynamics during microwave drying of cut tobacco.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 13

A smouldering cigarette on 10-Layer Whatman filter paper substrate: Steady-State Temperature Distribution.

Experiments have been conducted to measure the steady-state temperature distribution within a smouldering cigarette and a 10-layer Whatman No. 2 filter paper substrate, a configuration stipulated by ASTM E2187-02b Standard to test cigarette's ignition propensity. It was found that the temperature of the cigarette coal adjacent to the substrate was significantly lowered, causing ca. 27% reduction in the linear burn rate of the cigarette. A slight upward bending of the cigarette coal combined with a shrinking of the coal diameter provided limited natural buoyancy around the external coal periphery. This, combined with the oxygen diffusion through the cigarette paper, sustained the smouldering burn.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 15

Characterisation and influence of the porous structure of cigarette paper on CO deliveries.

The influence of cigarette paper on CO deliveries has been described in a number of studies. Two mechanisms play a major role: dilution and diffusion through the cigarette paper. The dilution is dependant on air permeability. The diffusion is dependant on the real porous structure of the paper. As the measurement of paper permeability does not allow to evaluate diffusion, cigarette papers having the same air permeability can give different CO deliveries.

The objective of this study was to have a tool that evaluates the capacity of the cigarette paper to diffuse CO, in order to better explain and control CO deliveries.

For this, we have studied different cigarette papers having identical air permeability, but giving different CO deliveries, which could only be explained by differences in the porous structure of the paper.

The methods we used to evaluate these papers were the following:

- Direct measurement of CO diffusion on unlit cigarettes,
- Diffusional Conductance Index based on electrical conductance of cigarette papers,
- Gas diffusivity measurement through the cigarette paper,
- Mercury porosimetry

Comparisons between the methods, their applicability and limitations will be given.

Simple ways of measuring paper porous structure will be proposed.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. IG 02

The regulation and governance of residues of plant protection products.

Numerous pesticides or plant protection products (PPP) are registered for use on tobacco around the world and the residues or metabolites of many of them can be detected in tobacco leaf samples. A number of national authorities and industry boards set maximum levels and standards for a wide range of these residues as well as for non-registered or obsolete PPPs on tobacco leaf or products. Residue levels for over 500 different residues exist in various forms of regulation.

To assist CORESTA Members and make them aware of the existence of these regulations the Agrochemical Advisory Committee has collated information from different sources on Maximum Residue Levels of PPPs from around the world. This effort has been supplemented by also gathering information on the global registration status of PPPs for use on tobacco and their application patterns. This paper outlines the main countries which have specific tobacco regulations for PPP residues, some of the underlying principles used to establish the levels and why in some cases there is little consistency between countries in the levels or residues. The global registration status information is useful for demonstrating the range of products and application rates which may lead to differences in residue levels in different countries.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 35

A practical approach to replicating human puff profiles on a mechanical smoking machine.

Considerable interest has re-emerged in the mimicking of human puff profiles as applied to mechanical smoking. The importance lies in investigating the correlation of smoke constituent uptake in epidemiological studies with the raw generation of such smoke constituents.

The capture of human profiles is an established principle with a number of commercial implementations marketed over the past twenty years.

A method of capture of profiles is described. The captured profiles have been used to define the puff profiles used by a specific mechanical smoking machine. A simple verification of the efficacy of the data download is employed to determine the practical limits on data resolution needed to faithfully reproduce the profiles.

Examples of captured human profiles are used to define the limitations of the system used and the effect on volume puffed on step resolution with reference to the ISO standard of 0.3 ml standard deviation on a 35 ml puff.

The current technology available for reproducing captured profiles is discussed and limits defined that allows a practical definition of “human like” profiles.

The content of existing mechanical puff profiles is discussed in relation to human like profiles.

The future direction of this technology is explored with reference to linear mechanical smoking machines.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 05

The effect of cigarette design variables on assays of interest to the Tobacco Industry: - 5) smoke aerosol properties.

The objective of this paper is to describe the measurement of the aerosol properties of mainstream tobacco smoke with respect to selected cigarette design variables. The experimental format has been described earlier (Case *et al*, 2005) for conventional dimensioned products involved using filter pressure drop, filter ventilation and paper permeability incorporating a statistically based central composite design. For the aerosol work, the Virginia blend type tobacco matrix has been used.

Four cigarettes of each type were smoked to a standard regime of eight 35 ml puffs of 2 second duration and at 60 second intervals. Measurements of particle diameter and number concentration were conducted after a 50:1 dilution step (Matter Engineering, CH) using a fast electrical mobility spectrometer operating at 10 Hz, with a measurement range from 10 – 1000 nm (DMS-500, Cambustion, UK).

A pattern of increasing concentration and decreasing diameter puff by puff was observed, although increased relative particle concentrations were often observed for the first puff. Median particle diameters for the Virginia blend cigarettes ranged from 190-270 nm, with a strong dependence on the degree of filter ventilation, implying residence time and coagulation are significant factors determining

size. A similar pattern of decreasing particle concentration with increasing filter ventilation was observed with a range from 2×10^{10} – 8×10^{10} particles per cigarette. The coefficients of variation for median diameter values are typically 2%; geometric standard deviations are typically in the range of 1.70 – 1.75.

Case, P.D. *et al.* The effect of cigarette design variables on assays of interest to the tobacco industry. 1) Experimental design and some initial findings on Hoffmann analyte yields

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 18

Sidestream tobacco smoke: novel measurement methods for particle size and concentration.

The objective of this work was to investigate the applicability of new rapid-response particle measurement techniques to sidestream tobacco smoke (SS) as it dilutes and ages. The new measurement techniques operate on the basis of corona-jet charging and electrical mobility measurement of the aerosol, offering improvements in operating range and time resolution over previous techniques.

In this work, an aged and diluted sidestream smoke was generated by machine smoking 10 cigarettes of 2 types in a sealed 20 m³ chamber. Environmental tobacco smoke (ETS), a combination of aged and diluted sidestream smoke with exhaled mainstream smoke was generated by 10 smokers, in a 120 m³ meeting room ventilated at 10 air changes per hour, using one of the cigarette types.

Particle concentration and diameter were measured by sequential 150s scans by a Scanning Mobility Particle Sizer (SMPS: TSI Model 3936L10) and at 1s intervals by a Condensation Particle Counter CPC: TSI Model 3022) and an Electrical Aerosol Detector (TSI Model 3070A).

Concentration data for SS shows a clear pattern of build-up and decay in the unventilated chamber with CO and particle decay half-times of 28 and 11.3 minutes respectively. Particle diameter increased from 100 to 150 nm count median diameter over a 45 minute period, consistent with particle accumulation.

In the ventilated room, particle concentrations and maximum diameters were lower, and the smoke concentration decay was more rapid.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 22

Development of an *in vitro* assay for the investigation of oxidative DNA damage induced by cigarette smoke.

Cigarette smoke contains a mixture of more than 4,000 chemicals, some of which may be involved in oxidative damage of biomolecules, including DNA (Pourcelot *et al.* 1999). As a method of investigating these potential effects on DNA, the modified *in vitro* Comet assay using the lesion specific enzyme Formamidopyrimidine glycosylase (Fpg), was combined with a novel Whole Smoke (WS) exposure platform.

The WS system was designed to expose cells at an air-liquid interface to mainstream cigarette smoke, Phillips *et al.* (2005). This system in combination with the Comet assay provides an insight into the induction of oxidative lesions by whole cigarette smoke.

Studies have shown that incorporation of a 16-20h recovery period for H292 cells exposed to various dilutions of WS, allows repair of the single strand breaks, leaving oxidative lesions, which can be revealed following Fpg treatment.

It is believed that this novel assay has potential for investigating the oxidative effects of tobacco smoke on cells and could be utilised in the investigation of new PREP technologies.

Pourcelot, S. *et al.* Free Radical Research 1999, 30:173-180.

Phillips *et al.* ATLA 2005 in-press

Patent WO 03/100417 A1

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 43

Puff-by-puff resolved measurements of cigarette smoke by Resonance-Enhanced Multi-Photon Ionization (REMPI) and Single Photon Ionization (SPI) - Time Of Flight Mass Spectrometry (TOFMS).

The application of soft photo-ionization mass spectrometry methods (PIMS) for cigarette mainstream smoke (MSS) analysis is demonstrated. Resonance-enhanced multi-photon ionization (REMPI) and vacuum ultra violet light single-photon ionization (SPI) were used in combination with time-of-flight mass spectrometry (TOFMS) for puff-by-puff resolved measurements of tobacco smoke. An optimized smoking machine with reduced memory effects of smoke components was constructed, which in combination with the REMPI/SPI-TOFMS instrument allows PIMS smoke analysis with a time resolution of up to 10 Hz. The complementary character of both PIMS methods is demonstrated. SPI allows the detection of various aliphatic and aromatic components in smoke up to about 120 m/z while REMPI is well suited for aromatic compounds.

The capability of the instrument coupled to the novel sampling system for puff-by-puff resolved measurements is demonstrated. Two main patterns of puff-by-puff behaviours are observed for different smoke constituents. The first group exhibits a constant increase in smoke constituent yield from the first to the last puff. The second group shows a high yield of the constituent in the first puff, with lower and constant or slowly increasing amounts in the following puffs. A third group can not be clearly classified and is a combination of both observed profiles. The results also illustrate that the specified quartz fibre filter (Cambridge filter) used for the separation of the smoke particulate matter from the vapour phase causes memory effects due to desorption of deposited material. This effect strongly influences succeeding puffs, which must be considered for puff-by-puff resolved interpretation of the gas phase. The feasibility of using the experimental system for intra puff smoke measurements is also shown.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 07

Gas Chromatographic Mass Spectrometric determination of Toscano cigar extracts aroma and characterization of their odor profiles by GC-Olfactometric techniques.

A comparative study was carried out on the volatile constituents of two brands of cigars: *Antico Toscano* and *Toscanello*.

Fifty-six volatile compounds were identified using High Resolution Gas Chromatography (HRGC) and HRGC-Mass Spectrometry. The volatile fraction is mainly characterized by nitrogen compounds (24 molecules) and organic acids (15 molecules).

Seventeen volatile compounds were detected only in *Toscanello* extracts, four of these compounds show a characteristic known aroma (butter, nuts, caramel, almond), while N-methylAcetamide was found only in *Antico Toscano* extracts.

Three multivariate statistical techniques were used to evaluate the experimental data: Analysis of Variance (Tukey test), Cluster Analysis (CA), Principal Component Analysis (PCA).

The Cluster Analysis (CA) shows the presence of two main clusters, one for each brand. The PCA confirms that the samples may be classified into two main groups on the basis of volatile compounds concentration, consistent with the grouping obtained during the CA.

The *Toscanello* samples are characterized by alkaloids, aldehydes (caramel, almond, wood flavor), some organic acid (flower, honey, cheese), 6-methyl-5-epiten-2-one and acetoin (butter).

The *Antico Toscano* samples are characterized by pyridines, pyrazine (coconut, nut, coffee flavor) and geranylacetone (rose, magnolia flavor).

Further studies, using different approaches, are in progress to determine the main odorants from the two brands of cigars.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 06

Volatile nitrosamines in cigarette smoke: a new sampling and analytical method.

Volatile nitrosamines (VNAs) in cigarette smoke are formed from decarboxylation of N-nitrosoaminoacids or pyrolytic nitrosation of secondary amines. Previous studies show that the levels of VNAs are much higher in sidestream smoke than mainstream smoke. Selective removal of VNAs from the mainstream smoke is achieved by cellulose acetate filters.

A new selective and sensitive method to collect and detect VNAs in tobacco smoke by gas chromatography coupled to a Thermal Energy Analyzer (GC-TEA) is under investigation.

The set up chromatographic method is able to identify N-Nitrosodiphenylamine (NDPHA), N-nitrosodimethylamine (NDMA), N-Nitrosopyrrolidine (NPYR), N-Nitrosodi-n-butylamine (NDBA), N-Nitrosodiethylamine (NDEA), N-Nitrosomethylethylamine (NMEA), N-Nitrosomorpholine (NMOR), N-Nitrosopiperidine (NPIP), N-Nitrosodi-n-propylamine (NDPA).

Ten cigarettes were smoked under controlled conditions. The mainstream and sidestream particulate phases were collected on a 44 mm Cambridge filter. The vapor phase of sidestream smoke was captured by two impinger solutions placed after the Cambridge pad. The impingers contained isopropanol solution and were cooled by ice-cooled water in a Dewar flask.

The total particulate matter trapped on the Cambridge filters was extracted with dichloromethane (DCM) in an ultrasonic bath for one hour. The pooled isopropanol solution and the dichloromethane extracts were concentrated up to 2 ml and analysed by GC-TEA.

Different extraction solutions (DCM, isopropanol, 1/1 DCM/Acetone solution) and different impinger solutions (isopropanol, 1% HCl) were compared. A clean-up procedure was tested.

The recovery rates for each solution were assessed by spike experiments of standard solution on both smoked and unsmoked filters.

NDMA and NPYR in sidestream and mainstream smoke were quantitatively determined. NMEA and NMOR were detected in sidestream smoke only.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 30

Determination of NNAL in smokers' urine by LC-MS/MS: a comparison of sample preparation procedures.

4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is a potent pulmonary carcinogen in rodents and may play a significant role as a cause of lung cancer in smokers. NNK uptake can be quantified by the analysis of two of its metabolites in urine, NNAL and NNAL-Glucuronide.

NNK is found only in tobacco products, therefore the presence of NNAL and NNAL-Gluc in urine is a specific biomarker of tobacco carcinogen exposure.

In this study our goal was to validate a simple procedure for sample preparation and successive determination of NNAL and its glucuronide in smokers' urine. Two different sample preparation methods were used and compared.

In the first method, based on a procedure found in literature, a single solid phase extraction step with cation exchange cartridges was used.

In the second method, specific molecularly imprinted polymer (MIP) solid phase extraction cartridges were used. MIPs are polymers with tailor-made selectivity for a target analyte. With these cartridges, NNAL can be selectively extracted from biological samples with high recoveries, allowing low limits of detection and reproducible results.

MS/MS detection following electrospray ionization was used to monitor the target analytes in both procedures.

The two methods were validated and compared by measuring free NNAL and total NNAL after enzyme hydrolysis of NNAL-Gluc with β -glucuronidase in 24 h samples of urine of five smokers and five non smokers.

Linearity, accuracy, precision, recovery and assay specificity were determined and will be reported.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 54

Application of a model reactor for efficiency testing of potential filter additives.

For the development of potentially reduced exposure products ('PREPS'), there is a demand for a quick and inexpensive pre-check methodology to access the effect of potential filter additives on certain constituents of mainstream cigarette smoke.

In this work a simple model reactor for this purpose is described that generally allows an estimation of the performance of filter additives towards selected Hoffmann Analytes (nitriles, aldehydes, ketones, aromatics) in mainstream cigarette smoke - without preparing cigarette test pieces.

The effect of a series of inorganic and organic polymers on the level of 20 vapour phase constituents is demonstrated for a monitor cigarette on a 12 mg/cig. tar level.

For this methodology, total cigarette smoke is passed through a layer of the respective filter additive and is sampled using a glass syringe.

An aliquot is subsequently analysed by GC-FID.

Physical characteristics of the materials are given and the effects observed will be briefly discussed on a molecular basis (e.g. adsorption and/or interaction of total cigarette smoke with reactive surface centres).

The methodology described is applicable for linear and rotary smoking machines.

Imperial Tobacco Group R&D, Hamburg, Germany.

NEWBURY J.P.

CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 33

Investigating the effects of alternative smoking regimes on selective filtration by cellulose acetate filters.

Previous work has shown that the selective filtration of a specific smoke component by cellulose acetate filters can be related to the component's solubility parameters and vaporization rate. That work was based on data collected under standard (ISO) smoking conditions. There is an interest in understanding how the selective filtration processes change with different smoking regimes, especially with respect to potentially reduced exposure products.

A change from the standard to a more aggressive smoke testing regime produces a complex mix of changes in the cigarette, including changes in the processes which affect delivery from the tobacco column to the filter and possibly the filter's selectivity. These changes lead to higher yields of most smoke components.

In this study, published data was used to compare smoke yields of Hoffmann analytes under two different test regimes for several commercial cigarettes. A 'Yield Ratio' is defined which relates the relative yield of an individual smoke component to the relative yield of nicotine-free dry particulate matter (tar). The yield ratio is analogous to the selectivity index.

This study showed evidence of a relationship between a smoke component's yield ratio and the properties that were shown to influence selective filtration. Also, for many of the smoke components, the yield ratio correlates well with ISO tar delivery for cigarettes included in this study. Furthermore,

the slope of the relationship between yield ratio and ISO tar delivery appears to be related to the component's physical properties.

Some initial hypothesis can be drawn from this study regarding the changes in yields of smoke components at intensive smoking regimes. Further work will be required to elucidate the specific role of selective filtration in these processes.

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OGDEN M.; HEAVNER D.; MORGAN W.; SEARS S.; RICHARDSON J.; BYRD G.

CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 32

Effect of creatinine and specific gravity normalization techniques on biomarkers in smokers' spot and 24-hour urines.

Renal excretion mechanisms are xenobiotic-specific; therefore, accurate exposure assessment requires an understanding of relationships of biomarker concentration and excretion rate to urine flow, specific gravity and creatinine concentration. 24-hour urine collection for xenobiotic exposure assessment is considered the “gold standard” procedure. Random spot-urine collection is convenient and minimizes subject compliance concerns but requires that normalization techniques be employed to account for diuresis and diurnal variation in biomarker excretion. This research examines and makes recommendations concerning normalization techniques and conditions under which spot-urine results most accurately reflect 24-hour urine results. Specific gravity, creatinine, and biomarkers were determined in smokers' spot and 24-hour urines. Normalization techniques were applied, variance-component analyses were performed to estimate variability, spot urines were pooled mathematically to simulate 24-hour urines and analyses of variance were performed to evaluate spot urines' ability to reflect 24-hour urine concentrations. For each biomarker concentration, log-linear relationships were observed with urine flow, specific gravity, and creatinine. For most biomarker excretion rates, log-linear relationships were observed with urine flow; creatinine, however, was unaffected by urine flow. The conventional creatinine ratio-normalization technique demonstrated greater variability (within-day, between-day and between-subject) than other normalization techniques. Comparisons of simulated 24-hour urines to spot urines suggest that spot-urine collection be performed only between 2 PM and 2 AM and that the modified specific-gravity-adjusted-creatinine ratio-normalization technique and the creatinine-regression normalization technique yield the best agreements between spot- and simulated 24-hour urine results.

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OTTE S.; INTORP M.

CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 39

Analysis of pesticides in tobacco by LC-MS/MS: specific matrix effects on quantification for different tobacco grades.

Pesticides are being used worldwide to protect agriculture products including tobacco against a wide range of pest and plant diseases. As a consequence of toxicity and persistence of these compounds, the monitoring of agricultural product quality is required. This way national residue regulations in different countries are met.

During the last years methodologies for the analysis of pesticide residues on tobacco have been established at different industry and contract laboratories and a large variety of procedures for extraction, clean-up, separation and detection were set up.

The common analytical method using GC and different detectors, e.g. NPD, ECD and MSD allows the determination of pesticide residues in tobacco at very low concentration levels.

However, in the last years a tendency to use more polar pesticides can be observed. Polar pesticides show lower persistence but higher toxicity than the non-polar ones. The well-established analysis by GC is less suitable for polar compounds; thus there is a demand for an alternative technique. As a consequence, the LC-MS/MS technique has been implemented by more and more laboratories.

The extracts for LC-MS/MS analysis are obtained by extraction, gel permeation chromatography (GPC) and dissolving in LC eluent. This sample preparation was first described in the well-known DFG S 19

method. In this work the determination of selected pesticide classes in tobacco will be demonstrated. Each analyte is characterized by two specific mass transitions.

The influence of tobacco matrix during ionization is a well-known challenge in pesticides analysis by LC-MS/MS. Different tobacco grades (air-cured, flue-cured and oriental) were analysed using matrix calibration. Specific matrix effects will be discussed.

Imperial Tobacco Group R&D, Hamburg, Germany.

PALANI N.; DINESH T.K.; PARTHIBAN R.; SURESH A.; RAMPRASAD H.N.; DHALEWADIKAR S.V.

CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 09

Quantification of volatile acids in tobacco.

Cured leaves of tobacco contain 3-11% of organic acids, which are known to add to smoke taste and flavour. Organic acids are present in free acid form as well as bound (sugar ester) form. Generally, flue-cured tobaccos contain higher quantities of high molecular weight fatty acids than burley or other air-cured tobaccos. High molecular weight saturated aliphatic fatty acids (C12 or higher) add a waxy, fatty and smooth taste to smoke. High molecular weight unsaturated fatty acids (C18:1, C18:2) add harshness to smoke. The low molecular weight C5 and C6 fatty acids contribute to flavour and aroma of cigarette smoke and are generally considered associated with oriental tobacco. Isobutyric, Isovaleric, valeric and 3-methyl valeric acid, short chain aliphatic fatty acids are associated with sweet, winy, buttery, nutty and fruity taste in cigarette smoke derived from oriental tobacco in the blend. Conventional methods provide quantification of free acid only and involve derivatization step. A simple and convenient method for the quantification of free and bound forms of acids has been developed. Quantification of 3-methyl valeric acid present as sugar esters is done by transesterification followed by GC. Volatile acids in tobacco present in free form are quantified by dynamic headspace coupled with GC. Recoveries for all the five acids are above 90%.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 07

Quantification of solanesol content in tobacco.

Solanesol, a C45 isoprenoid is useful both as a medicine by itself and also as an essential intermediate used to synthesize a side chain component of coenzyme Q₁₀ (Ubiquinone Q₁₀), an important cardiac drug. Although solanesol is present naturally in some other plants, tobacco is the only known commercially important source of solanesol. It was isolated from tobacco for the first time in 1956. Typically, Solanesol is quantified by HPLC with C18 column and acetonitrile / methanol mobile phase with concentration gradients. In the new method, solanesol and its esters were extracted from tobacco with the help of Soxhlet apparatus using hexane, followed by saponification. The saponified material is further analysed using HPLC equipped with C8 column and using constant ratio binary mobile phase consisting of acetonitrile and methanol. The limits of quantification (LOQ) and limits of detection (LOD) also have been optimized and recoveries of more than 90% could be achieved.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 29

Biomonitoring of nicotine and cotinine in saliva to assess human smoke exposure: a new validated analytical methodology.

Nicotine, the most important tobacco alkaloid, is quickly metabolized in humans. Cotinine is the main metabolite of nicotine and it is obtained by oxidation of the pyrrolidine ring. The presence of nicotine, cotinine and other metabolites in biological fluids (e.g. saliva, urine, serum, etc.) makes them very

important biomarkers for measuring smoking status, mainstream tobacco smoke (MSS) and environmental tobacco smoke (ETS), in both active and passive smokers.

Tobacco smoke was found to be a human carcinogen, therefore the simultaneous analytical determination of these compounds in biological fluids could be very significant to correlate tobacco smoke related toxic effects and risks.

Previous studies have found that nicotine and cotinine in biological fluids could be the most specific and sensitive biomarkers for tobacco smoke exposure status; however, as the half-life of nicotine ($t_{1/2}=1-2$ h) is much shorter than those of its metabolites, analysis of cotinine ($t_{1/2}=18-20$ h) provides advantages for monitoring the parent compound.

In this study, we developed and validated a new analytical method for the extraction and the determination of nicotine and cotinine in saliva.

After ultra-filtration on Amicon membranes, saliva samples were extracted and an aliquot was analyzed by GC-MS/EI equipped with a spherical ion trap analyzer. Quantification was performed by selected ion monitoring (SIM) mode combined with MS/MS experiments.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 13

A test piece for determining dense end offset repeatability and reproducibility using the microwave method.

The use of microwave devices for the measurement of mean density and dense end offset has become commonplace in the analysis of cigarette production. However a practical problem remains in relating the measurements obtained to the readings from such devices. Measurements are generally “noisy” and lacking in definition. The user relies upon the computer algorithms to provide a reading of dense end offset that may not be evident from visual inspection alone.

The dense end offset can be defined as the distance from the cigarette end where the density profile first equals the density mean. In practice the density varies in such a way as to instil a degree of uncertainty in the measurement apparatus. It is often difficult to distinguish the effect of measurement error on the given dense end figure.

The paper describes the development of a simple test piece that allows repeatable measurement of the density profile using the microwave method. The test piece is based upon creating a rod made of segments of distinctly different densities but with a known mean density. In this way the dense end offset can be accurately predicted and measured for confirmation. Detail of the construction is provided.

The repeatability of the microwave measurement used in two commercially available devices is established and the effect of temperature on the test piece is explored. Consideration is given to the robustness of such a method / test piece.

Future use of a test piece to provide a verification method for moisture content as measured by the microwave method is discussed.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 08

The effect of cigarette design variables on assays of interest to the Tobacco Industry: smoking behaviour studies.

The objective of this poster is to describe the smoking behaviour and dosimetry studies carried out to determine the effect of design changes, in particular increase in paper permeability at a range of cigarette pressure drops (58 – 125 mmWG). The experimental format for conventional dimensioned products involved using filter pressure drop, filter ventilation and paper permeability incorporating a statistically based central composite design.

Two smoking behaviour and dosimetry studies were carried out. The five products chosen from the Virginia blend type tobacco matrix for the first study comprised two ~6 mg products and three ~10 mg

products only differing in their paper permeability. Three pairs of ~6 mg, ~7 mg and ~9 mg products from the 1:1 Mix of the Virginia and Burley matrix, at different pressure drops differing only in their paper permeability were selected for the second study.

At a total cigarette pressure drop of ~87 mmWG, increasing paper permeability from 35 to 69 CU had little or no effect on the consumers' smoking behaviour; the dose obtained and perceived sensory intensities of ~6 mg & ~10 mg products in study 1. However, increasing the paper permeability from 33 to 75 CU at a total cigarette pressure drop of 58 mmWG resulted in relatively higher tar but significantly higher nicotine delivery in study 2. There were no significant differences in the smoking behaviour or perceived mean sensory scores between the two products.

Increase in paper permeability from 33 to 75 CU at higher cigarette pressure drop \geq 87 mmWG had little or no effect on the consumers' smoking behaviour; the dose obtained and perceived sensory intensities of ~6 mg & ~9 mg products.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 27

***In vitro* bioactivity of combustion products from twelve tobacco constituents.**

The toxicity of smoke is often being studied using cigarette smoke condensate (CSC) samples. In this project, twelve chemical components of tobacco leaf, representing about 50% of its dry weight, were selected. They included carbohydrates, amino acids, proteins, polyphenols and carboxylic acids. They were individually combusted and the bioactivities of their combustion products (i.e. condensates) were assayed using three *in vitro* tests. The mutagenic potencies were assessed with the Bacterial Reverse Mutation / Ames test (*Salmonella typhimurium* TA98 and TA100). The induction of chromosomal damage was determined with the Micronucleus test (IVMNT), using V79 Hamster lung fibroblast cells. The Ames test and IVMNT were conducted both in the presence and absence of rat liver microsomal S9 fraction, to assess metabolites' toxicity. The Neutral Red Uptake cytotoxicity test (NRU) was also conducted using V79 cells. Ames data confirmed the mutagenic potencies of combustion condensates obtained from nitrogenous compounds (amino acids and proteins) of tobacco. The IVMNT showed that precursors of phenols in smoke (chlorogenic acid and lignin) exhibited significantly higher levels of toxicity compared to other tobacco components. While S9 activation amplified the Ames response to combustion products, it significantly inhibited the toxic response measured with the IVMNT. NRU data demonstrated the increasing cytotoxicity induced following longer exposure time to condensates from nitrogenous and phenolic components. These toxicological data characterize the toxicity of the combustion products of twelve tobacco constituents. They also underline different mechanisms of toxicity as revealed by the different bioassays' responses.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 09

Predictivity and variability of *in vitro* bioassays routinely used to estimate the toxicity of cigarette smoke.

An amendment to the Tobacco Act is currently being introduced in Canada. This will require all brands of cigarettes sold in this country to be subjected to a set of three *in vitro* assays on an annual basis. The purpose of the amendment is to monitor potential changes in the toxicity profile of cigarette smoke over time. The tests will also be used to compare toxicity of standard and reduced ignition propensity cigarettes, which are being introduced at the end of the year as a result of additional legislation.

The bioassays are very similar to those used by the *In Vitro* Task Force in their recent evaluation of relative toxicity of four types of cigarette tobacco, i.e. Ames Bacterial Mutation, Neutral Red Uptake

Cytotoxicity and the *In Vitro* Micronucleus Tests. However, presumably to allow comparability of data between laboratories and across time, the Health Canada methodology is very specific in most aspects of study design.

To determine the predictive accuracy of the assays, it is vital to determine the inherent variability within each assay. For example, if the apparent activity of a brand of a given brand of cigarettes increases by 25% between testing in 2005 and 2006, is that due to changes in the cigarette, or is it more likely due to the variability of the biological assay.

We have examined variability of each bioassay using 2R4F Kentucky reference cigarettes smoked under intense smoking conditions (volume 55 ml; interval: 30 sec.). The influence of the following factors has been examined:

- Variability between cultures and plates
- Replicate smokings
- Experiment to experiment variation
- Length of time in storage
- Activity relative to concurrent positive and negative controls

Coefficients of variance have been calculated for each factor. These results, together with historical control information, should be taken into account when considering the relevance and reliability of the results of individual assays.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 08

Protein removal from tobacco.

Proteins and amino acids are precursors of numerous nitrogen compounds in smoke together with alkaloids. The reduction of proteins content in tobacco can contribute to reduce certain Hoffmann's analytes in smoke such as N heterocyclic compounds (quinolines, N-heterocyclic amines), aromatic amines, HCN.

Proteins level in tobacco (up to 20%) is highly variable and depends on genotypes, tissue, cultural and curing conditions. The curing of tobacco significantly modifies proteins content and properties. Approximately 50% of proteins are hydrolyzed or degraded during harvesting and curing. Most of the remaining proteins have a lower solubility than in green tobacco.

An overview of potential methods to remove proteins in tobacco will be presented.

Proteins removal methods can be divided in two classes: proteins removal from fibres materials and from soluble extract. Proteins in fibres are strongly linked or imbedded into the tobacco matrix and their extraction is more difficult.

Fibres proteins may be removed by proteins solubilisation (alkali, surfactant, salting-in treatments) or by proteins hydrolysis (enzymatic treatments). Soluble extract proteins may be removed by different techniques such as precipitation, adsorption and filtration.

Factors such as pH, ionic strength, temperature, reactant concentration can improve protein solubility or precipitation.

Efficiency of different treatments for proteins removal in fibres and water extracts will be reported.

LTR Industries, Le Mans, France.

RICHTER A.(1); MAUNDERS H.(2); MASSEY E.D.(1); PATWARDHAN S.(1)

CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 21

A toxicogenomic study of primary human lung epithelial cells exposed to whole cigarette smoke.

Cigarette smoking is strongly associated with the development of lung cancer and chronic obstructive pulmonary disease. In order to investigate the potential mechanisms of smoke toxicity we have performed a transcriptomics study of the effects of whole cigarette smoke on human lung epithelial cells.

Primary bronchial epithelial cells from a series of human donors were seeded onto cell culture inserts and grown at an air-liquid interface for 3 weeks to induce mucociliary differentiation. The cells were then exposed to either filtered air, or whole smoke diluted 1/50 or 1/300 in air (smoke/air, v/v) at an air-liquid interface for 1, 6 and 24 hours using an exposure system developed by BAT. These doses of smoke were previously established to be minimally toxic and sub-toxic to the cell cultures. Total RNA was extracted, processed, labelled and hybridised to Affymetrix HG U133 Plus 2.0 arrays. Raw data were scaled using Affymetrix GCOS software and analysed for robust changes using Genespring. Data on genes consistently up-regulated or down-regulated by smoke in all or most of the donor cell lines, intra- and inter-donor variability and a functional interpretation of the response will be presented.

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ROSE N.

CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 10

Instrument for automatically air permeability test of banded papers.

Regarding the New York guidelines for meeting ignition propensity standards the cigarette must contain two banded zones on the paper surrounding the tobacco column. These cigarettes must pass the ASTM E2187-02B test that demands that 75% of the tested cigarettes should extinguish by it self under the test conditions.

The air permeability of the banded zones plays an essential role in the design for this cigarettes.

The poster presents an automatically operating instrument for the measurement of air permeability of the banded zones and the base paper according the ISO standard 2965. The instrument offers particular ease of operation. This is evident not only in the setting of the paper width and the fully automatic paper transport but also in the fully automated measuring sequence and the efficient structure of the unit. The operator can choose whether the paper feed is controlled by a band detection sensor or by a fixed, stated value.

A microprocessor control unit regulates the measuring sequence for single measurements or series of measurements as well as the documentation and statistical evaluation of the results.

Borgwaldt Technik GmbH, Hamburg, Germany.

ROSE N.

CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 11

New smoking machine family RM20/200.

This poster gives an overview about a new family of rotating smoking machines for the different applications within the tobacco industry. A special machine is needed for every single purpose. To cover this, a new product line, based on one standard rotary unit was designed.

The RM20H is a semi automatically operated smoking machine for special analytes and R&D tasks that support the range of mainstream traps that are required by the Hoffmann analysis list. Therefore the machine has a compartmentalized smoke trap area, designed to accommodate standard filter pad traps, XAD-4, electrostatic and multi impinger traps, cooling baths etc..

The RM20B is a completely manually operated smoking machine that is newly designed for the same field of application.

These machines are supplemented by the RM20S, a smoke generator for *in vitro* research.

On top of the line is the new designed RM200A. A fully automatically working smoking machine for routine analytical smoking. This machine combines ease of use with a maximum of throughput.

All machines are designed to smoke according to the ISO 3308 standard and to the requirements for intensive smoking. Individual smoking parameters are also possible.

Borgwaldt Technik GmbH, Hamburg, Germany.

ROSE N.

CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 25

Smoke generator for *in vitro* research.

In close cooperation with BAT Southampton, a smoking machine was developed, which generates cigarette smoke for *in vitro* research.

The machine called RM20S is based on the rotary principle of the RM20H. It is able to smoke four different brands simultaneously under ISO conditions. The machine dilutes the smoke and forwards the dilution into the cell chambers. The fully automated process offers the possibility to supply the cell chambers with smoke for several hours.

The general advantage of the rotating principle is that all necessary tools like loader, lighter and so on are only needed once. The cigarettes are subsequently moved to the respective position. The smoke ring offers four cigarette holders, which are moved into five positions: loader, lighter, smoke position, diluting, glow detection and extraction.

The machine is equipped with four independent stepper motor driven syringes with an adapted 5/4-direction-control-valve for selecting the flowpath. The syringes and valves are designed such that residual does not effect the function. The hood and air flow control allows to smoke under ISO 3308 conditions.

The machine is controlled by several micro processor systems which operate to the master slave principle. For operation the machine is equipped with a terminal with display, keyboard and printer.

Borgwaldt Technik GmbH, Hamburg, Germany.

SCHERER G.; HAGEDORN H.W.; URBAN M.; ENGL J.; RIEDEL K.

CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 31

Influence of smoking charcoal filter tipped cigarettes on the uptake of benzene and 1,3-butadiene.

Charcoal (CC) filters of cigarettes are known to significantly reduce a series of volatile constituents in mainstream smoke including carbonyls, benzene and 1,3-butadiene. We performed a randomized, cross-over, 2-week brand-switching study with 39 smokers. Twenty (20) of the subjects smoked cellulose acetate (CA) filter tipped cigarettes during the first week of the study, the remaining 19 subjects smoked CC filter tipped during the first week. In the second week, the subjects switched to the corresponding brand with the other filter type with similar smoking machine-derived tar and nicotine yields. Efficiency of the CC filters of the cigarettes used in the study was tested with a cell-free glutathione (GSH) consumption assay. Daily cigarette consumption, carbon monoxide in exhaled breath, salivary cotinine and urinary nicotine equivalents (molar sum of nicotine plus 5 major metabolites) did not significantly change when switching to the cigarettes with the other filter type. Urinary excretion rates of monohydroxybutenyl-mercapturic acid (metabolite of 1,3-butadiene) and S-phenyl-mercapturic acid (metabolite of benzene) were significantly lower when smoking CC compared to CA filter tipped filter cigarettes.

We conclude that smoking CC filter tipped cigarettes does not change the uptake of carbon monoxide and nicotine when compared to CA filter tipped cigarettes with similar tar and nicotine yields, but leads to a significantly reduction in the exposure to toxicological relevant smoke constituents such as 1,3-butadiene and benzene.

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SEXAUER W.

CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 12

A method to classify the offset of different filter rod measurement systems.

Calibration of pressure drop instruments does not necessarily lead to the same values on the various instruments. Under some circumstances systematic differences between the artificial standards and results of real filter rods (obtained on different instruments or from different locations) could be observed.

In some cases we have been able to identify an offset when comparing measurement apparatuses with a higher number of filter rod samples. From this observation we learned that a balancing of test results from different measurement systems can be achieved by using strictly selected filter rods with particular properties.

Environmental conditions are reflected in the results of selected filter rods stored close to the instrument. The actual impact of the instrument can be evaluated with rods stored under ISO conditions. Cross checks between different parties can be performed more reliably.

The experiences and advantages of this method are lined out in this presentation.

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SHEPPARD J.; WARREN N.D.; CASE P.D.

CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 02

The effect of cigarette design variables on assays of interest to the Tobacco Industry: - 2) prediction of smoke and Hoffmann analytes using two different modelling methods.

The objective of this paper is to describe the development of various statistically based methods for the prediction of mainstream Hoffmann analyte yields with respect to selected cigarette design variables. The experimental format being that described earlier (Case *et al*, 2005)

Two different statistically based modelling approaches were developed. The first model used the cigarette design variables of filter pressure drop, filter ventilation and paper permeability with a constant (Virginia) tobacco blend. The second model involved a proportionality input to enable incorporation of the other blend types namely Burley, Oriental and a 1:1 mix of Virginia and Burley. This method used a reference set of measurements to predict changes in smoke and Hoffmann analyte yields.

From the multiple regression techniques carried out the majority of the models produced using the two different methods gave good prediction models for Hoffmann analyte yields. From these models, estimates of Hoffmann analyte yields can be made for other cigarette products.

The prediction models produced are only as good as the analytical data that is provided. The more reliable the analytical data is the more useful the prediction models will be for Hoffmann analyte yields. Other cigarette design parameters can be explored and these would give further prediction models.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 26

Effect of cigarette filters on the *in vitro* toxicity of mainstream smoke.

Charcoal is used widely in cigarette filters to primarily reduce the amount of certain vapor phase constituents of tobacco smoke.

The objective of this study was to compare *in vitro* toxicity of cigarette mainstream smoke from the charcoal filtered cigarette with that of Kentucky Reference Cigarette 2R4F. In this work, we used 3 types of cigarettes included non-filtered 2R4F cigarette (NFC), 2R4F reference cigarette (RFC), and carbon-filtered 2R4F cigarette (CFC) which was made from original 2R4F by replacing with an acetate filter containing charcoal. The mutagenicity of the cigarette smoke condensate (CSC), which was collected in Cambridge filter pad or cigarette filter from these cigarettes, was assessed in *Salmonella typhimurium* TA98 and TA100 with S9 metabolic activation. In addition, the cytotoxicity of both the CSC and the gas/vapor phase (GVP), which was bubbled through in phosphate-buffered saline in a gas wash-bottle, was determined using a neutral red uptake assay.

Results for mutagenicity of CSC extracted from Cambridge filter pad were CFC > RFC > NFC. Corresponding values for CSC from cigarette filter were RFC > CFC. The mutagenic potential of CSC from the cigarette filter was significantly less than that of the Cambridge filter pad on the equal CSC basis. With regard to cytotoxicity when calculated on an equal CSC basis, the cytotoxicity of both the CSC fraction and GVP fraction from the CFC was lower relative the 2R4F cigarette (RFC).

These results will be discussed with the chemical composition of mainstream smoke from 3 types of cigarettes.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 17

The transfer characteristics of scopoletin on to cigarette paper.

Some compounds in tobacco leaf are transferred to cigarette paper by moisture. The purpose of this study was the identification and quantitative analysis of the high polarity transferred compounds on to cigarette paper. The transferred materials were extracted with distilled water using ultrasonic extraction at 50 °C. The extract was purified with column chromatography, filled with C18. The purified fractions were collected and re-purified in the same manner. The purified fraction was analyzed by gas chromatography / mass spectrometry (GC/MS). Four polyphenol compounds, benzothiazole, Salicyl alcohol, Formyl nornicotine, 4-Cumyl phenol, and Scopoletin peaks were observed in the GC/MS profile. Among them, scopoletin had very strong fluorescence intensity with very low concentration at ex 342 nm / em 460 nm. This property of scopoletin enabled a quantitative analysis of transferred materials on the cigarette paper. Scopoletin was quantified by HPLC equipped with a fluorescence detector, and fluorescence profile was measured by fluorescence spectrophotometer.

From this study, we found that the moisture in cigarette was directly proportional to transferred material amount on cigarette paper.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 51

The transfer ratio of some ester flavors in cigarette.

Some ester flavors were injected in cigarette and then analyzed with simultaneous distillation and extraction equipment and GC and GC/MS to observe the transfer rate to cut tobacco, filter tip, mainstream smoke and the cigarette butt. The results show that: 1) the reserved condition impact the transfer ratio of the ester flavors after the flavors being injected in cigarette. 2) The ester flavors with low molecular weight and low boiling points tend to dissipating. Airproof is the precondition to prevent ester flavors losing. 3) With well airproof, the lower molecular weight and boiling points ester flavors show more flavor transfer to filter tip. Otherwise their transfer ratio is lower. 4) Ester flavors with high molecular weight and boiling points have a high ratio into cut tobacco and a low ratio into filter tip. There is a balanced distribution between filter tip and cut tobacco. 5) No objective flavors stay in cigarette paper and filter tip when they are departed rapidly after being injected in cut tobacco. 6) The ester flavors with low molecular weight and low boiling points have low hold ratio in cut tobacco. Except cinnamate ester flavors, the other ester flavors' hold ratio in cut tobacco, transfer ratio to filter tip and mainstream smoke increases as their molecular weight and boiling point is increasing. 7) The cinnamate ester flavors hold in cut tobacco are closely up to 100%, the transfer ratio to filter tip, mainstream smoke are high. 8) The ester flavors' transfer ratio to butt is low.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 45

Pyrolysis single-photon ionisation time-of-flight mass spectrometry (Py-SPI-TOFMS) for the discrimination of three tobacco types.

Pyrolysis single photon ionisation/time-of-flight mass spectrometry (Py-SPI-TOFMS) has been applied to differentiate between three major tobacco types, viz Burley, Virginia, and Oriental, respectively. SPI is known as a soft ionisation technique that allows fast and comprehensive on-line monitoring of a large variety of aliphatic and aromatic substances with no or only few fragmentation of the molecule ions. Tobacco samples were pyrolyzed at 800 °C in a nitrogen atmosphere. The resulting pyrolysis off-gas contained signals from more than 70 masses in a mass range between 5 m/z and 170 m/z. Mass spectra obtained were analyzed by Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) to distinguish between different tobacco types. Prior variable reduction of the data set was carried out by calculation of the Fisher-Ratios. Results achieved give information about chemical composition and characteristics of the pyrolysis gas from each tobacco type and enable conclusions on plant cultivation. Based on LDA, a model for tobacco type recognition of unknown samples was established, which was cross-checked by additional measurements of each tobacco type. Furthermore, first results on the recognition of tobacco mixtures based on Principal Component Regression (PCR) are presented.

Recognition of different tobacco types is possible by only analyzing a few key substances without prior treatment of the samples. First measurements of tobacco mixtures provided promising results and reveal the potential of Py-SPI-MS for applications in various fields. Latest improvements of the ionisation technique such as the development of electron-beam pumped rare-gas excimer VUV-lamp systems for SPI, which replace costly laser instruments; incorporation of quadrupole mass spectrometers, and commercially available pyrolyzer devices including automated sampling systems would lead to a wide applicable technique, whose operation requires a minimum of expenditure of time.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 55

Evaluation of injection techniques for the analysis of tobacco smoke vapor phase.

The analysis of volatile organic compounds (VOCs) has been performed applying such methods as direct injection, cold trapping, solvent trapping or adsorption-thermal desorption. The usefulness and availability of such methods is often limited due to the complicated equipment operation required, which may lead to poor reproducibility. Over the last decades, several equipments capable of automatic sampling and solvent-free injection of VOCs have been developed and used in various analytical fields, including the vapor phase of tobacco smoke. The aim of our study was to evaluate these methods and to select the appropriate system that we subsequently used for the analysis of the tobacco smoke vapor phase.

To this end, we compared a custom-made direct injection system with a sampling loop against a preconcentrator (7100A, Entech) and an adsorption-thermal desorption system (TDS2, Gerstel). The vapor phase of tobacco smoke was collected in a Teddler® bag and injected into the gas chromatograph (GC) in each case. The data obtained using each system were analyzed for sensitivity, reproducibility sample stability over repeated analyses and range of volatility of the detected compounds.

As a result, the sensitivity of the Entech and TDS systems was determined to be higher, because of the large volume of the sample that could be injected, the adsorption-desorption mechanism and the cryo-focusing. Highly diluted samples proved more stable, which constitutes another advantage of the Entech and TDS systems. On the other hand, some semi-volatile compounds were detectable only with TDS. In this report we discuss the data obtained and recommend to use a particular system for the analysis of tobacco smoke vapor phase according to its intended purpose.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 20

The influence of age and storage conditions on the activity of carbon in cigarette filters.

The use of activated carbon in cigarette filters is now well established and the ability of activated carbon to reduce the levels of compounds in cigarette smoke is well known. It is also known that during storage the carbon in a cigarette filter tends to undergo a reduction in activity. The factors governing this change in activity are not yet fully understood. An acceptable measure of the activity of carbon to cigarette smoke is the retention of vapour phase compounds. The current study looks at the effect of age and conditions of storage on the activity of carbon in a cigarette filter.

Carbon filter samples have been stored under four different conditions. These are temperature, -18 °C, low water content, temperature 4 °C, low water content, temperature 22 °C, 60%RH and temperature 35 °C, 75% RH. For each storage condition the vapour phase retention of the carbon has been measured as a function of sample age for up to six months ageing. Other important filter properties such as the hydrolysis of the plasticiser have also been studied as a function of storage temperature and age.

The effect of storage conditions on the change in activity of the carbon on storage as measured by the retention of major vapour phase aldehydes, ketones, hydrocarbons and acrylonitrile determined using standard ISO smoking and GC-MS analysis will be discussed. The rates of plasticiser hydrolysis as measured by the generation of acetic acid in the filters are also discussed. Recommendations for optimum storage conditions are given.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 10

Analysis of saccharide in tobacco by High Performance Liquid Chromatography with Evaporative Light Scattering Detection.

A high performance liquid chromatographic method (HPLC) with evaporative light scattering detection (ELSD) for the determination of saccharide in tobacco was developed. Two of monosaccharide: glucose, fructose, and two of disaccharide: sucrose and maltose in tobacco were analyzed. The tobacco products after drying were smashed and filtered by 40 meshes sieve. The sample powder was extracted by 80% ethanol under the condition of high pressure (1500 PSI) and comparatively high temperature (80 °C) through Accelerated Solvent Extractor, and then the protein in extracted solvent was precipitated by PbAc₂ and filtered out. The final solvent after volume adjustment was analyzed through HPLC with evaporative light scattering detection. The experiment data indicated that the method had the detection limit of less than 0.003 mg/mL, the recovery fell between 93% and 104%, and the RSD (n=8) less than 5% in which glucose, fructose and sucrose less than 3%. Applying the method for determining above 4 saccharide in 15 cigarette products and 46 different type of tobacco samples were determined. The results showed that the applying ELSD in saccharide determination of tobacco products had the advantage of stability, rapidity and exactness.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 11

Analysis of performance characteristics of cigarettes sold in New York State that meet ignition strength regulations.

As of June 28, 2004, only cigarettes that pass the ASTM E2187-02B ignition strength test more than 75% of the time have been allowed for sale in New York. In this paper we will explore the changes in design of selected commercial cigarette that resulted in this decreased ignition strength.

We sampled 14 cigarette brands from the New York market and compared their performances with the same brands sold in other states that were not designed to meet the New York regulations. This study

was not meant to be an exhaustive analysis of all cigarettes sold in New York, but rather cigarettes were chosen to represent a broad range of design options.

In many cases it was determined that cigarette manufacturers had to make changes to the paper design to be compliant with the New York regulation. All cigarettes purchased in New York state met the ASTM ignition strength standard, but there were differences in cigarette free-air smolder rate and deliveries.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 14

Influence of alginate banded paper on selected Hoffman analytes.

All cigarettes sold in New York State as of June 28, 2004 have had to meet cigarette ignition propensity test criteria. Most of the cigarettes meet the regulation by coating bands of a film former on the paper to reduce the paper permeability in selected regions. The physical properties and composition of these bands may influence smoke deliveries and particularly Hoffman Analytes (HA). The performance characteristics of the low ignition propensity (LIP) cigarettes have been discussed in a companion paper.

One of the technologies uses sodium alginate to form such bands on otherwise conventional cigarette paper. Cigarettes using these designs pass NY regulations for cigarette ignition propensity. What happens to cigarette smoke deliveries and Hoffmann Analytes (HA) when these papers are used to improve LIP performance? This study will explore the influence of banded cigarette paper using sodium alginate bands on selected HA deliveries. A limited study showed that certain HA were reduced in test cigarette with alginate paper compared to the control. This presentation will discuss the findings.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 44

Puff-by-puff resolved tobacco smoke particulates analyzed by Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (GCxGC TOFMS).

The potential use of GCxGC TOFMS in the analysis of puff-by-puff resolved tobacco smoke particulate matter collected on Cambridge filters is discussed. GCxGC-TOFMS is a technique suited to study complex samples consisting out of thousands of compounds. The separation capabilities of GCxGC were already shown by various researchers. The combination of this technique with a mass spectrometer provides an even more sophisticated means of analysis. The GCxGC provide good separation of compounds allowing compound mass spectra with low matrix noise, this again aids deconvolution algorithms to identify compounds with better library matches. However, the data sets are getting enormously complex, hundreds of peaks are revealed with each having a deconvoluted mass spectrum. In the tobacco smoke profiles analysed more than 10 000 peaks can be identified. To process this amount of compounds routines capable of handling huge amounts of data need to be used. A classification procedure based on GCxGC retention times and the fragmentation patterns is suggested. With this classification procedure it is then possible to group compounds with some certainty into substance classes. The substance classes can then be compared with one another in different puffs. Other methods of comparing and contrasting the smoke particulate profiles collected from the different puffs like direct comparison of chromatograms and chromatogram subtractions are also demonstrated.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 24

Validation of a new smoking machine for the exposure of 96 well cell culture plates to 'native' cigarette mainstream smoke aerosol. Course of action and some *in vitro* toxicological data.

In order to generate toxicological data of cigarette aerosol different smoke exposure systems have been designed the last years. These machines should simulate smoke exposure conditions comparable to human exposure.

Since 1999 we have been optimising the BT020 smoking machine for *in vitro* testing. Easy handling in practice and high throughput of test cigarettes has been a very important point in the development process. The BT020 is a single port smoking machine directly connected with a novel cell exposure system for Multiwell Cell Culture Plates. A purpose-built smoke distribution device disperses the cigarette smoke across the Multiwell Plate. All wells of the plate are provided with separate smoke distribution and suction ducts. The 'intelligent' computer controlled smoke dilution system allows for precise and rapid dilution of freshly generated cigarette smoke or vapour phase. The machine is applicable for testing cigarettes yielding various amounts of 'tar'.

The smoking machine was validated by the uniformity of 'tar' deposition in the wells and by the reproducibility of toxic effects in the Neutral Red Uptake assay (NRU) with human Hep-G2 liver hepatoma cells. The proliferation tests with differently ventilated cigarettes showed reproducible data. Taking into account different smoke dilution factors, toxicological data from 'high tar' cigarettes can be compared with data from 'low tar' cigarettes.

An additional advantage is the simple preparation of cells prior to smoke exposure. For 96 Multiwell Plates in particular we use a simple method to assure that cells are only covered by a thin layer of fluid like lung epithelial cells. The cells show different toxic reactions with 'whole smoke' and vapour phase of cigarettes, indicating a significant contribution of the particulate phase.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 03

The effect of cigarette design variables on assays of interest to the Tobacco Industry: - 3) tobacco blend type.

The objective of this paper is to examine the potential relationships between various tobacco blend components and Hoffmann analyte yields across three distinct lamina tobacco blend styles which are Virginia, Burley (uncased), and Oriental; additionally a 1:1 mixture of and Virginia/Burley was also included.

For each blend style various cited Hoffmann blend precursors e.g. nitrogen, sugar, polyphenols, etc; and Hoffmann analyte yields were measured using a constant cigarette design, which has been described previously (Case *et al*, 2005).

Correlations were found with these studies that indicate possible relationships between blend chemistry components and Hoffmann analytes in smoke, which, in the majority of cases agreed with published literature. Other analysis techniques included cluster analysis, by which precursors are grouped together; additionally linear regression analysis also identified blend components that allow prediction of certain Hoffmann analyte yields from tobacco precursors. The analysis techniques can also be used to illustrate that certain Hoffmann analyte yields can be correlated to more than one source of precursor. These relationships potentially indicated certain blend components that could, in future, be included with other cigarette design parameters in mathematically derived equations to predict Hoffmann analyte yields in mainstream smoke.

However the correlations obtained should not be interpreted as 'cause and effect', in that a specific blend component is definitely a precursor of a particular Hoffmann analyte.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 28

Primary studies on the correlation between adverse biological effects and important harmful chemical components of smoke in cigarette safety evaluation.

Objects: This study was in order to research the correlation between adverse biological effects and important chemical components of cigarette smoke, offer benefits to the evaluation on lower hazard cigarette and definition of the target decreasing hazard of cigarette.

Methods: The toxicologic detected method and the chemical analytical method were united to evaluate cigarette smoke safety for 22 brands cigarettes. The toxicologic methods, including assessment of genotoxicity, cytotoxicity and acute toxicity of TPM, resulted in five biological indicators: MR value, SCE frequency, micronucleus frequency, death rate of cell, and death rate of mouse. The chemical analytical method consisted of five compounds: tar, nicotine, CO, TSNA, BaP. Besides, correlation between toxicological date and chemical date were examined using qualitative correlation analysis and quantitative multi factors regression analysis.

Results: There were some correlation between adverse biological effects and important chemical components of cigarette smoke, TSNA was the most important factor influencing MR value, SCE frequency and death rate of cell ($P < 0.01$), but micronucleus frequency, as well as death rate of mouse, was no relative to tar, nicotine, CO, TSNA and BaP ($P > 0.05$). However, the correlation conclusion needs to be further validated by larger samples.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 41

On-line puff-by-puff smoke monitoring and aroma gas phase analysis of cigarette paper and tobacco.

At WFT Research, on-line puff-per-puff analysis of mainstream smoke is performed using a Airsense 2000 mass spectrometer. By employing a specially designed 3-way valve, the measurements can be directly carried out on a RM1+ Borgwaldt smoking machine. Sensitive real time monitoring of gaseous and volatile compounds, such as CO, CO₂, NH₃, aldehydes, hydrocarbons, aromatic compounds, and other Hoffmann type analytes can thus be carried out. The instrument can be operated by using both ion molecule reaction ionization and electron impact ionization.

In addition, we also employ the Airsense instrument for automated aroma monitoring of cigarette paper using the headspace technique. This enables the distinction and quality control of different grades of papers based on the liberation of volatile and eventually odour relevant compounds. In combination with our routine GC-MS technique, the results are used for aroma characterization and classification of cigarette papers. Furthermore, this technique can also be used for the distinction and classification of tobaccos.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPTPOST 14

Determination of HCN content in mainstream cigarette smoke using constant flow analyzer-new indication reaction and better performance.

Isonicotinic acid and 1,3-dimethyl barbituric acid were applied as the indication reagents for the determination of HCN content in mainstream smoke using constant flow analyzer (CFA). The recovery

rate of HCN was above 99% and the relation coefficient was 0.9996 in the linear range 6.2×10^{-2} —15 ($\text{mg} \cdot \text{L}^{-1}$). The influence of buffer solution and reaction conditions on this method was also studied. Possible interferential effects caused by 14 kinds of anions and 15 kinds of metallic ions were surveyed to prove the stability of this determination method. Compared with pyridine-pyrazolone indication system, isonicotinic acid / 1,3-dimethyl barbituric acid indication system provided easier operation, better stability, higher sensitivity and better repeatability, and was more environment friendly.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 56

Influence of nitrate, nitrite and nicotine on Tobacco Specific N-nitrosamines in mainstream smoke.

In this paper, the relationships between tobacco-specific N-nitrosamines in mainstream smoke and their precursors were studied. The nitrate, nitrite, nicotine were added respectively into the cigarettes, the total particular matter was collected according to the standard method. The treated samples were analyzed quantitatively by GC-TEA according to the internal standard method. The relationships between NNN, NAT, NAB, NNK, total level of tobacco-specific N-nitrosamines and nitrate, nitrite, nicotine were estimated. The results showed that the nitrate, nitrite and nicotine were the important precursors of the tobacco-specific nitrosamines. With the amounts of the added precursors enhanced, the level of tobacco-specific nitrosamines increased. There were significant relationships between these precursors and tobacco-specific N-nitrosamines.

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CORESTA Meet. Smoke Sci.–Prod. Techno Groups, Stratford-upon-Avon, 2005, abstr. SSPT 42

Application of single-photon ionisation time-of-flight mass spectrometry (SPI-TOFMS) with an electron beam pumped excimer light source for the on-line detection of organic compounds in mainstream smoke.

Single-photon ionisation (SPI) with vacuum-ultraviolet (VUV) light allows an efficient and soft ionisation of organic compounds yielding mass spectra with no or only few fragment peaks. Selectivity is provided via the ionisation potential (IP), as only compounds with an IP lower than the photon energy can be ionised. A compact and mobile quadrupole mass spectrometer (QMS) system using a novel electron beam pumped rare gas VUV lamp for SPI has been demonstrated recently to be a useful tool for on-line analysis of organic trace compounds in complex gaseous mixtures such as cigarette main stream smoke. This concept is expanded by combining a gateable VUV lamp with a time-of-flight mass spectrometer (TOFMS) providing a further development step resulting in even faster mass analysis due to the generation of in principle a complete mass spectrum with every single VUV light pulse. The interfacing of the VUV light source exhibiting a repetition frequency of 50 Hz with the TOFMS is described and the SPI-TOFMS system is characterised. On-line detection limits down to 50 ppb for benzene, toluene and m-xylene (BTX) as well as acetone were achieved. The instrument is well suited for continuous measurements of aromatic and aliphatic trace compounds and can thus be used for on-line monitoring of trace compounds in dynamically fluctuating gaseous mixtures. Puff-by-puff resolved measurements of cigarette main stream smoke illustrate the applicability of the technique for on-line detection of various Hoffmann analytes.

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