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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 08

Characterisation of a cigarette smoking machine designed for air-liquid interface exposures: an inter-laboratory study

The Borgwaldt RM20S smoking machine generates, dilutes and delivers whole cigarette smoke for *in vitro* testing at the air-liquid interface (ALI). The RM20S, in combination with BAT's exposure chamber (patent publication no. WO 03/100417 A1), enables direct exposure of *in vitro* cell/tissue cultures to whole cigarette smoke at the ALI, from 30 minutes to 3 hours duration. The machine smokes up to eight cigarettes simultaneously into eight independent syringes and dilutes this smoke consistently, in a dynamic range.

A rigorous evaluation of two RM20S machines from two independent locations was conducted; the laboratories being in Montréal and Southampton. Smoke dilution and precision were compared and characterised as an essential step to validating these machines. A methane gas standard was used to assess syringe dilution precision. Dilutions of 1:1000, 1:500 and 1:193 (gas:air, volume:volume) were tested and measurements were compared between labs and syringes. Quantification of methane for each syringe was made using the heated flame ionization detector (FID) total hydrocarbon analyzer. No statistically significant differences (P >0.05) were observed in the lower dilution ranges of 1:1000 and 1:500 between labs. For all three dilutions, combined residual standard deviation was between 6.3% and 17.3%, R2 >0.98 and error between 4.3% and 13.1%.

Values obtained from these experiments were within acceptable limits (RSD <10% and R2 >0.95) indicating that the equipment can reliably dilute accurate doses of whole smoke for ALI exposure and dosimetry studies. Despite some noticeable variability, all syringes were still consistent across locations. Furthermore, having assessed the precision of the smoking machine in two different laboratories and with different operators provides us with even greater confidence in the reliability of equipment and of smoke dose. These results suggest the Borgwaldt RM20S system provides a reliable and repeatable method for generating and delivering whole smoke to *in vitro* ALI exposure systems.

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Mentholated tobacco products: Options for the tobacco industry following potential regulatory restrictions

In 2009 the Family Smoking Prevention and Tobacco Control Act gave the FDA legal authority to regulate tobacco products with the objective of protecting public health and reducing the harm to the public from products causing nicotine addiction and disease.

Under this authority, one of the first acts of the FDA was to ban flavoured cigarettes, except for those containing menthol, which account for about 28% of the US market. Additionally, the FDA created the Tobacco Products Scientific Advisory Committee (TPSAC) mandated to review and report on the impact of the use of menthol cigarettes on public health. The TPSAC submitted their report to the FDA in March this year finding that evidence from studies did not conclude that smokers of menthol cigarettes have a greater disease risk than smokers of non-mentholated cigarettes, which is in line with scientific evidence provided by the tobacco industry. However, the TPSAC recommended that "removal of menthol cigarettes would benefit public health in the US" largely, but not only, based on the belief that mentholated cigarettes encourage smoking initiation in minors, adolescents and certain ethnic groups.

Although the FDA did not ban mentholated cigarettes or other mentholated tobacco products, immediately following the TPSAC report, there is no time limit to their deliberations and a future ban could still be imposed.

This poster presentation will briefly consider an independent view of some possible options open to the tobacco industry following potential regulatory restrictions on the sale of mentholated cigarettes and other mentholated tobacco products.

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Cytotoxic effects of different tobacco preparations on human peripheral blood mononeucleocytes

Acute exposure to cigarette smoke or its components has been known to trigger diverse cellular effects, such as oxidative stress, DNA damage and cell death in a number of cell culture models. However, available data regarding the potential cytotoxic effects of Smokeless Tobacco (ST) lacks consensus. This research was initiated to investigate the relative biological effects of ST, and whether ST elicits differential cellular / molecular responses compared to the combustible tobacco preparations.

Total particulate matter (TPM) and whole smoke conditioned medium (WS-CM) were prepared from 3R4F reference cigarettes, while 10% 2S3 reference moist tobacco extract was prepared in artificial saliva with enzymes (ST/CAS). Human peripheral blood mononuclear cells (PBMCs), isolated from donors (who are non-consumers of tobacco products), were used as a model to examine the systemic effects of exposure to the tobacco preparations and nicotine, in short-term cell culture assays. Since PBMCs consist of a large variety of cell subsets, which differ in abundance, the following major PBMCs subsets viz., T helper (CD4) cells, cytotoxic T (CD8) cells, monocytes (CD14 cells) and NK cells (CD56 cells) were evaluated. Cytotoxicity (assayed by cell death, expressed as EC_{50} values) and DNA damage (γ-H2AX) were determined by flow cytometry.

Corresponding EC_{50} values, normalized for nicotine content of the tobacco preparations, suggest that combustible tobacco preparations induced markedly higher cytotoxicity as follows: WS-CM ≥ TPM >> ST/CAS > nicotine. WS-CM and TPM similarly induced time-dependent cytotoxicity in PBMCs. While all three tobacco preparations induced detectable levels of DNA damage in a dose-dependent manner, the combustible tobacco preparations were significantly more potent than ST/CAS. Further, the leukocyte subsets exhibited differential cytotoxicity when exposed to combustible tobacco preparations in the following order: CD4 > CD8 > monocytes > NK cells. In contrast, the treatment with nicotine and ST/CAS was less cytotoxic and did not exhibit cell type selectivity.

Thus, the data presented herein suggest that in terms of nicotine content/unit, the combustible tobacco preparations are markedly more cytotoxic than ST/CAS or nicotine to human PBMCs.

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Comparison of mouth level exposure to tar and nicotine in smokers of normal and superslim King Size cigarettes

This study evaluates whether a superslim cigarette design format has any influence on mouth level exposure (MLE) to tar and nicotine compared with more conventional designs. Most King Size (KS) cigarettes have a circumference of about 25 mm and are 83-84 mm long, whereas the King Size Superslim (KSSS) is the same length, but has a circumference of 17 mm. Filter analysis studies to estimate MLE to tar and nicotine were conducted in Romania and Russia.

A single KSSS commercial product was chosen to represent each 1, 4 and 7 mg ISO pack tar level. Comparisons were made with a single product, at each ISO pack tar level of KS cigarettes with both activated carbon filters (King Size Carbon - KSC), to assess the effect of format on MLE, and cellulose acetate filters (King Size Non-carbon - KSNC) to assess the effect of filter carbon on MLE. A target population of 50 to 70 male and female smokers of each product (subjects), aged 21 to 50 years, were recruited in each market. The subjects gave written informed consent before commencing the study.

The Russian smokers tended to obtain higher MLE compared with the corresponding Romanian smokers.

For MLE to tar no significant differences were found between the Romanian smoker groups within any ISO pack tar. In Russia, the KSC smokers obtained higher estimates than the KSSS smokers at 4 mg and 7 mg ISO pack tar.

For MLE to nicotine, the KSC 1 mg and KSSS 1 mg smokers obtained similar estimates within each country. Within the 4 mg and 7 mg pack tar groups the KSNC smokers tended to obtain lower estimates compared with the other smoker groups corresponding to lower blend nicotine levels.

In conclusion, no systematic MLE differences were found resulting from these design elements. Confirmation would require scientifically matched controls.

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Producer compliance costs for Good Agricultural Practices in the U.S.

Most purchasers of U.S. tobacco now require producers to comply with some set of good agricultural practices (GAP). Each manufacturer or leaf dealer has their own version of GAP, but in general there are three categories into which GAP can be categorized: 1) crop management, 2) environmental stewardship, and 3) labor management. While there are differences, individual company GAP is similar to or based on GAP standards recommended by CORESTA. Adoption of GAP by both U.S. and other manufacturers is often driven by liability concerns, actions of advocacy groups (particularly in labor management and environmental stewardship), and regulatory action at the tobacco product level (e.g. in the U.S. regulation by the Food and Drug Administration). Producer compliance costs are manifested in direct costs such as upgrades to labor housing or installation of conservation practices. However, a large component of compliance costs is indirect costs, which is often difficult to measure. In economic terms these costs are categorized as transactions costs. The purpose of this study is to examine and, where possible, quantify costs associated with adoption of GAP. Where costs are not quantifiable, the relative importance and impact on management is examined. For example, increased record keeping is required for most practices. This task is either carried out by the producer or employees assisting with farm management. Increased record keeping often does not result in hiring of a new employee, but may increase time required for management or reallocation of human resources. Preliminary results indicate that the incurrence of such transaction costs is one factor in farm structural change, particularly increasing farm size, on U.S. farms. There seem to be significant economies of scale associated with many of the transactions costs due to adoption of GAP.

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Using a structural model based on a class of generalized covariance criteria, to explore the generation process of smoke compounds

Our aim is to explore a structural model: several variable groups describing the same units are assumed to be structured around latent dimensions that are linked together through a linear model that may have several equations. This type of model is commonly dealt with by methods assuming that the latent dimension in each group is unique. However, conceptual models generally link concepts that are multidimensional. We first propose a general class of criteria suitable to measure the quality of a Structural Equation Model. This class contains the covariance criteria used in PLS Regression methods and the Multiple Covariance criterion of the SEER method. All criteria in the class must be maximized under a unit norm constraint. This maximization is used within a general algorithm named THEME (Thematic Equation Model Exploration), which allows us to extend the multiple covariance criterion in two ways: 1) The criterion is generalized so as to take into account the structural strength of a component in a more flexible way, including multiple covariance, but also variable-bundle-oriented measures; 2) It is also extended to models having any number of dependent / predictor groups.

We have applied THEME to model the production of Hoffmann compounds from 52 physical and chemical variables describing 19 cigarettes. The variables were partitioned into 7 thematic groups, linked through 2 equations structural model. Equation 1 models Hoffmann compounds smoke contents under intense smoking from the characteristics of the cigarette (combustion process), and equation 2 models Hoffmann compounds smoke contents under ISO smoking regime from those under intense smoking regime and characteristics of filter under ISO smoking conditions (Filter ventilation process). THEME allowed us to separate the complementary roles, on Hoffmann compounds, of: tobacco quality, tobacco type, combustion chemical enhancers or inhibitors, filter retention and ventilation powers. It yielded a complete and robust model having accuracy within reproducibility limits.

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Determination of r&R variability for the expression of smoke constituent level per unit amount of smoke nicotine

Limits on the levels of selected mainstream smoke constituents per milligram of nicotine (nicotine ratio) have been proposed as an option for regulatory compliance of cigarettes. However, these limits must come with tolerances in order to avoid misleading conclusions on certain brands. To establish tolerances, it is essential to know the accuracy of the applied methods and for that, the widely used method is to perform a collaborative study in order to determinate the repeatability and reproducibility. This paper describes the different collaborative study processes used for accuracy measurement according to the dataset (raw data, ratios with or without paired measurement). Use of unpaired measurements for computing ratios highlights the difficulties of repeatability and reproducibility assessment. Indeed, due to analytical constraints the measurements of smoke components and smoke nicotine are not always carried out simultaneously (unpaired measurements), therefore the ISO 5725 statistical approach can be inappropriate. For example, accuracy of NNN yield per milligram of nicotine (from a recent CORESTA Special Analytes Sub-Group joint experiment) calculated using the ratios generated with permutation strategies, showed the wide range of estimated repeatability and reproducibility: 112% and 77%, respectively under ISO smoking regime for the 1R5F cigarette.

Therefore, as the accuracy of nicotine ratio expression, with unpaired measurement, cannot be clearly determined using the basic method for the determination of repeatability and reproducibility defined by ISO standard, some strategies will be suggested to approximate these uncertainties.

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Effect of sugar content on acetaldehyde yield in cigarette smoke

The relationship between cigarette blend sugar and acetaldehyde formed in its smoke is a matter of current regulatory interest. Some tobacco scientists have used data by Phillpotts to illustrate that cigarette smoke yields of acetaldehyde are independent of inherent or added sugar levels in the parent tobacco blends. Some tobacco control advocates (O'Connor and Hurley) have recently interpreted the data differently. In this work we re-evaluated the Phillpotts data and also similarly evaluated our own chemistry data across cigarettes representative of our full portfolio. Using the Phillpotts's data, O'Connor and Hurley recently applied a multivariate analysis to determine the relationship between acetaldehyde in smoke and sugar in tobacco taking into account the NFDPM yields. Indeed, a multivariate analysis enables the partial effect of different factors, especially product design, to be taken into account. With this analysis normalized for NFDPM, the authors concluded that the sugar-acetaldehyde relationship had a coefficient of correlation of 34%. However, the authors decided to use only the data which come from filter cigarettes whereas it would be more relevant to consider the full dataset and to add in the model a qualitative factor with two modalities (FP: filter and plain cigarette). Moreover, the difference between cigarettes sold in different countries has not been taken into account in their model although Phillpotts mentioned the specific behaviours of Italian and French brands. Therefore, the multivariate analysis used by O'Connor is incomplete. In order to avoid any misleading conclusions, we decided to carry out a multivariate analysis (General Linear Model (GLM)) taking into account all the factors (Sugar, NFDPM, FP and Country). Contrary to the O'Connor conclusion, our results have shown the sugar content does not have a significant impact on aldehyde yield.

In a second step, we have examined some data sets obtained in our laboratory between 2001 and 2010 on 99 commercial brands from the EU market: 12 from Eastern Europe (Poland, Hungary, Ukraine); 34 from France; 14 from Germany; 8 from Spain and 31 from the UK. In our studies, a GLM analysis with the sugar factor nested in the country factor has shown no effect of sugar content on acetaldehyde yields whatever the country. Moreover, an analysis taking into account the blend style has also highlighted no relationship.

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Ranges of mainstream smoke formaldehyde yields from contemporary cigarette products

Objectives: Formaldehyde is an IARC Group 1 carcinogen present in cigarette smoke. Concerns over its potential contribution to smoking related diseases have led to proposals for limits on the ratio of formaldehyde to nicotine yields in mainstream smoke as measured under Health Canada Intense (HCI) machine smoking conditions (Burns *et al*., 2008). However, there is surprisingly little published data on formaldehyde yields from contemporary cigarettes; of the thousands of cigarette brands on sale globally there are around 150 recent published ISO Formaldehyde smoke yield values (and fewer HCI smoke yields) covering relatively few countries. The current work was conducted to substantially enhance the size of the available yield database, and to provide greater clarity on the ranges of formaldehyde yields commonly obtained from contemporary cigarette products.

Method: Formaldehyde smoke yield data were assembled from published data (with control products and duplicate brands removed), and British American Tobacco (BAT) measured products. ISO yield data were analysed by quantile regression using the SAS statistical software.

Results: 700 ISO Formaldehyde yields have been assembled from the following sources: UK benchmark (25 brands), Health Canada 2004 survey (60), Health Australia (15), Counts *et al*. 2005 (48) and BAT measured brands (552 brands) covering 69 geographical areas. Quantile analysis of ISO data has provided median, quartile, and major percentile values for formaldehyde yields at ISO tar level from 1 to 15 mg. There was insufficient HCI formaldehyde yield data to conduct quantile analysis, however sufficient data is available to conduct an analysis of the ratio of HCI formaldehyde to nicotine yields. The limited HCI ratio dataset was found not to be normally distributed, therefore an empirical cumulative density analysis was conducted on the ratio values.

Conclusions: This work extends considerably the database of available formaldehyde smoke yield data. Statistical analysis approaches have provided numerical frameworks that allow for greater clarity on the range of formaldehyde yields commonly found with contemporary cigarette products.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 52

The enhanced filter removal efficiency by novel ventilation system composed of dual perforation

Generally when designing cigarettes, the effect of filter removal and ventilation is used to ensure the target content of tar and nicotine. Cigarette design includes acetate tow selection to realize filter pressure drop and control of tip perforation to realize air dilution. In this study, a novel dual ventilation system was introduced to enhance filter removal efficiency. This system is composed of normal mouth end perforation (mouth side) and additional perforation located in the opposite side (tobacco side).

Experiments were performed in two ways. At first, to compare mono and dual ventilation systems, we manufactured a series of mono and dual perforated cigarettes with a range of ventilation levels. We analyzed physical properties, smoking constituents and the filter removal efficiency. The results show that the dual perforated cigarette has higher filter removal efficiency and a lower decrease of unencapsulated pressure drop (UPD) when compared with mono perforated cigarettes.

Secondly, mono and dual perforated cigarettes were compared under the same ventilation levels. The cigarettes having same ventilation were manufactured by decreasing ventilation of mouth side as the tobacco side ventilation is increased and analyses were performed in the same way. This time the results show that dual perforated cigarettes under the same ventilation show enhanced filter removal efficiency and increased UPD.

From these experimental results, we can infer that this dual ventilation system can be applied to cigarette design to enhance filter removal efficiency and to control physical properties.

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Isolation, screening and identification of Bacillus with high ability of protein degrading and aroma enhancing from tobacco leaf surface

By taking the advantage of high-temperature resistance of *Bacillus* and adopting special separation technology, a strain of *Bacillus* with a high ability for protein degradation and aroma enhancement was isolated and screened from the surface of flue-cured tobacco leaves. The ratio (HC value) of diameter of protein degrading circle to colony diameter was up to 5.50 and protease activity up to 25.616U. The microbial agent prepared from the screened *Bacillus* was used to ferment tobacco leaf. The determined degradation rate reached 29.32%, which provided a reference for improving the quality of tobacco leaf with microbial fermentation.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 16

Spectroscopic investigations into the acid-base properties of nicotine at different temperatures

The nicotine molecule contains two separate and distinct ionisable moieties both centred on nitrogen atoms. In aqueous solution nicotine can exist in three states of ionisation: under acidic conditions (low pH) it will be present predominantly as the doubly charged cation, at intermediate pH it will be present as a singly charged cation, while in alkaline solvation nicotine will be present predominantly as an un-ionised, neutral molecule.

The more acidic ionisable moiety is the one located on the aromatic pyridyl ring; under acidic conditions the doubly charged cationic nicotine species is in equilibrium with the singly charged species and at around pH3 (pK_{a1}) the two cationic species are equally prevalent. An equilibrium also exists between the singly charged cation of the pyrrolidyl moiety and the neutral molecule - at around pH8 (pK_{a2}) both these entities will be equally prevalent. These equilibria are affected by the temperature of the aqueous solution.

The aim of the present study was to investigate the acid-base equilibria of nicotine in aqueous solution using circular dichroism and UV absorbance spectroscopy and to determine the pK_a values of nicotine at temperatures between 20 and 40 °C. The salient results were as follows:

$pK_{a1} = 2.96 \pm 0.02$, $pK_{a2} = 8.07 \pm 0.01$ at 20 °C
$pK_{a1} = 2.85 \pm 0.02$, $pK_{a2} = 7.89 \pm 0.01$ at 25 °C
$pK_{a1} = 2.84 \pm 0.05$, $pK_{a2} = 7.83 \pm 0.03$ at 30 °C
$pK_{a1} = 2.74 \pm 0.03$, $pK_{a2} = 7.57 \pm 0.02$ at 40 °C

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Results of the 2010 CORESTA Part Filter Method Ring Trial

The Part Filter Method (PFM) is used to estimate smokers' mouth-level exposure to nicotine and tar. Previously in 2006 the CORESTA Smoking Behaviour Sub-Group commissioned a ring trial of the PFM in which participating laboratories completed the trial according to their own protocol; nine laboratories returned data. A learning from this study was that laboratories that utilised standardised calibration smoking regimens were more consistent. In the protocol for the 2010 study, participants were requested to use a set of defined calibration smoking regimens. Two cigarette types (ISO NFDPM 1 and 10 mg) were forwarded to the laboratories together with machine smoked test filter tips (cut to 10 mm lengths) from each cigarette type which had been smoked under one of two smoking regimens, more intense (65 mL puff volume, 1.5 s duration, 50 s frequency) or less intense (40 mL puff volume, 2.0 s duration, 60 s frequency) giving rise to four exposure levels. Laboratories analysed the test tips using their own procedures for extraction and analysis. Participants completed three independent replicates of estimates each consisting of three individual samples. The results were reported to the study co-ordinator for collation and statistical evaluation.

In the estimation of nicotine yields from tip nicotine, determined in twelve laboratories, across the four levels, the repeatability (within laboratory precision) was 20% and the reproducibility (variation between laboratories) was 54%.

In the estimation of tar from tip UV, determined in nine laboratories, across the four levels, the repeatability was 22% and the reproducibility was 57%.

In the estimation of tar from tip solanesol determined in six laboratories across the four levels, the repeatability was 23% and the reproducibility was 65%.

The test tip extracts from the less intense regimen of the 1 mg product was close to the limit of quantitation for many laboratories, this partly accounts for the high variation in repeatability and reproducibility.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 02

Analytical method validation steps - benefits, limitations and an alternative approach

In the framework of the growing regulations regarding tobacco products, interest is expressed in a wider product characterisation in terms of contents and emissions. This leads to the need for new analytical method developments. One of the last steps of the development consists in a proper in-lab validation in order to measure the performance of the method and evaluate its accuracy, as required by ISO17025 for accreditation. Method validation processes have been discussed and standardised in some areas, for food or water quality analyses for example, while there is currently no standard generally agreed for validation of methods related to tobacco products. The processes followed are then usually largely inspired from existing standards developed in other different areas. The aim of this paper is to present firstly the steps for a proper analytical method validation (linearity characterisation, limits of detection and quantification determination etc.) and to underline the corresponding benefits & limitations. Secondly, an alternative approach based on accuracy profile of a method is presented.

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Determination of a wide range of N-nitroso-compounds in smokeless tobacco through the use of tandem mass spectrometry coupled to gas phase chromatography

The number of N-nitroso compounds of regulatory interest in smokeless tobacco has increased in recent years. The US FDA Tobacco Products Scientific Advisory Committee (TPSAC) proposed list includes eight volatile or semi-volatile N-nitroso compounds as well as the four traditional tobacco nitrosamines (TSNA). Unfortunately, the widely used LC-MS/MS method for the analysis of TSNA is not easily adapted for the analysis of volatile N-nitroso compounds given the low molecular weight and volatility of these compounds. The objective of this study was to develop a single analytical method capable of determining all the compounds in one analytical method. Gas chromatography coupled with tandem mass spectrometry was used to measure the compounds. In brief, ground tobacco samples were extracted with alkaline methylene chloride, filtered and injected without further sample preparation. The N-nitrosoamines were separated on an Agilent 7890 using an HP-5MS column with subsequent detection on an Agilent 7000 QQQ tandem mass spectrometer. The mass spectrometer was operated in positive chemical ionization mode using ammonia and all compounds were determined in daughter ion mode (MRM). In general, all compounds gave excellent levels of detection and were less than 1 ng/mL. Calibration and other analytical details will be presented for all compounds. Two compounds, N-Nitrosodiethanolamine (NDELA) and N-nitrososarcosine (NSAR) proved difficult to analyze and required significant method development work. We will present additional information on the determination of these compounds.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 18

Determination of hydrazine in smokeless tobacco products

Hoffmann and co-workers (Liu, Schmeltz & Hoffmann, Anal. Chem., 1974, 46 (7), pp 885-889) have previously reported the determination of hydrazine, which has been classified by IARC (IARC Monograph 71, 1999) as Group 2B (possibly carcinogenic to humans), in tobacco and tobacco smoke. However, no information is available in the literature on the concentrations of hydrazine in contemporary smokeless tobacco products (STPs).

In order to establish whether hydrazine is present in contemporary STPs a method was developed for its analytical measurement. Hydrazine was extracted from the STPs by agitation with methanol/0.1M hydrochloric acid. After centrifugation, an aliquot of the supernatant was derivatized using pentafluorobenzaldehyde to form decafluorobenzaldehyde azine (DFBA). The DFBA was subsequently partitioned into hexane and reduced in volume by rotary evaporation, before quantification by GC/MS using a Varian 3800/Saturn 4D instrument fitted with a 30 m × 0.25 mm DB5 capillary column.

The method was validated by the assessment of linearity of response, recovery, accuracy and precision. The recovery of hydrazine from five different STPs fortified at three different concentrations (0.0265 - 0.53 µg/g) ranged from 64% to 105%, and the relative standard deviation of 5 replicate analyses ranged from 3.2% to 12% across this concentration range.

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Standards, regulations and testing of biodegradable polymers

Although various standardization working groups have been working for many years on the issues of biodegradability and compostability, some people often are still confused about what these terms really entail. Yet, the European norm EN 13432 as well as the comparable ASTM specifications D6400 and D6868 have strict procedures with clear criteria and have been used successfully for several years now. Based on this experience also, an ISO norm was published in 2008 : ISO 17088 and another one is moving to ISO stage (ISO DIS 18606).

This presentation starts with a discussion on the European norm EN 13432 on compostability of packaging and making the distinction between biodegradability and compostability. Compostable materials must meet certain requirements with regard to composition (mainly heavy metal limits). Full biodegradation is required within a period of 6 months. Also disintegration must be achieved according to strict limits for a test material at a maximum thickness (more than 90% of input material must pass 2 mm). Finally, compost quality is evaluated via physical-chemical analyses and plant toxicity tests. The various requirements are discussed in detail.

Further, a description is given of systems to put this norm into practice (certification and logos); experiences obtained after 10 years of usage of this norm in various European countries; future outlook; and finally a comparison with similar norms in North America and Japan.

In the last part of the presentation, other environments are discussed, sketching the similarities and the differences that are mostly related to temperature but also microbial population that is present. More in particular, biodegradation in home composting, in soil and in water is discussed with again focus on test methods as well as criteria. Also a brief overview will be given on the standards existing in these different fields.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 41

Study on the correlation between the free amino acids and sensory properties of flue-cured tobacco

The relationship between the free amino acids and the sensory properties of flue-cured tobacco leaves was studied by partial correlation analysis and canonical correlation analysis in this paper. The correlation was significant. Arginine, methionine and tyrosine were found to positively impact, and valine negatively impact the sensory properties based on partial correlation analysis. Arginine, methionine, tyrosine, glycine were revealed to contribute to the canonical correlation between free amino acids and sensory properties including concentration, aroma volume, smoke strength, abundance, sweetness, offensive taste and irritation. Increasing the content of arginine, methionine, tyrosine, glycine, isoleucine within a moderate range could improve the sensory properties but with negative impact when beyond this range.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 14

Nicotine Pharmacokinetics: Comparison of a cigarette, loose snus, pouched snus and nicotine gum

In a consumption survey of Swedish snus users, we found that users of loose snus consumed a greater weight of tobacco/day than users of pouched snus. To investigate possible differences in the nicotine absorption kinetics following use of loose snus compared to pouched snus, we conducted a randomised cross-over pharmacokinetic study in Sweden.

Six products were included in the study: 2 pouched snus products (approximately 9 mg and 14 mg of nicotine/portion), 2 different portion sizes of loose snus (approximately 9 mg and 23 mg of nicotine/portion), a cigarette (1 mg ISO nicotine yield) and a nicotine gum (nicotine replacement therapy, 4 mg of nicotine/piece). All the products were commercially available in Sweden at the time of study initiation (January 2010).

The 20 volunteers who completed the study were daily snus users and occasional smokers for at least 6 months prior enrolment. They attended a Clinical Research Unit in Sweden on 6 occasions to test 1 product per visit. The concentration of plasma nicotine was determined at intervals for two hours during and after product use.

All of the snus products were used for a period of 1 hour, which corresponded with the median t_{max}. The median t_{max} for the nicotine gum was 45 minutes (usage time: 30 minutes) and 7 minutes for the cigarette (usage time: approximately 5 minutes). Systemic nicotine exposure, based on $AUC_{0\text{-}tlast}$ and C_{max} increased with increasing nicotine content for both types of snus, although increases in exposure were sub-proportional to the increases in nicotine content. All of the tobacco products gave higher systemic nicotine exposure than the nicotine gum. The nicotine exposure was similar for both of the 9 mg nicotine pouched and loose snus portions with no statistically significant difference seen between these two products, suggesting that product form had no effect on the absorption kinetics of nicotine.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 09

Validity of the 44 mm Cambridge Filter Pad for trapping Total Particulate Matter in excess of 150 mg

Current ISO smoking standards limit the maximum quantity of Total Particulate Matter (TPM) that should be trapped on a 44 mm Cambridge Filter Pad (CFP) to 150 mg.

When smoke methods were first developed in the 1950s, cigarettes had smoke yields much higher than those commonly available today. A consequence of this was that to produce a TPM yield of 150 mg, at a rate of about 8 puffs per cigarette, required approximately 40 puffs using only five cigarettes.

Nowadays products have much lower yields and for 150 mg of TPM to be collected using, e.g. a 1 mg/cig product, approximately 1000 puffs may have to be taken.

In addition, new more intense smoking regimes, e.g. Health Canada Intense, have been adopted and these are known to generate significantly more water, which may also affect pad integrity.

To characterise the trapping efficiency of the CFP with contemporary cigarettes, an investigation has been conducted to examine the capability of 44 mm CFPs to trap quantities of smoke up to and beyond the ISO standard limit.

Incremental numbers of 1R5F and 3R4F University of Kentucky reference cigarettes were smoked onto separate 44 mm CFPs to obtain a range of TPM from approximately 50 to 400 mg/pad. The range of the number of puffs taken for 1R5F and 3R4F were 160 to 1300 and 40 to 320 respectively.

Nicotine, water, TPM, nicotine free dry particulate matter (NFDPM) and carbon monoxide (CO) were measured. A comparison of the results was made between TPM up to the ISO limit of 150 mg/pad and those up to approximately 400 mg/pad.

Under ISO regime smoking, the trapping efficiency of the pad was found to be constant up to 150 mg/pad. When the ISO limit was exceeded, the yields per cigarette of TPM, nicotine and water were found to slightly reduce. The reduction begins to appear around 220 mg per pad. In addition, the deviation from the standard ISO yield was greater for the ventilated, lower yielding 1R5F cigarette than that of the 3R4F product. However, the ratios of CO and NFDPM to TPM did not appear to have been compromised when the ISO limit was exceeded.

A further observation was the pressure drop (PD) of CFPs increasing slightly as the TPM reached 400 mg, however this was well below the 0.9 KPa recommended by ISO.

In conclusion, this work has shown that, with contemporary cigarettes during ISO regime smoking, the CFP retains its integrity up to the current ISO standard and beyond. However, there is a small reduction in the yield of some smoke analytes when the ISO TPM limit is exceeded on a 44 mm CFP. Further work is needed to clarify whether the composition of this reduced yield TPM changes above 150 mg/pad during ISO smoking and whether the 44 mm pad behaves in a similar manner under the Health Canada Intense smoking regime.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 30

Biomarkers for the smoking-related exposure to 1,3-butadiene

1,3-butadiene is a Class 1 carcinogen ('carcinogenic to humans') according to the International Agency for Research on Cancer (IARC). This chemical is also on a WHO recommended list of toxicants in mainstream smoke of cigarettes for mandated lowering. It occurs at various workplaces, in combustion exhausts, ambient air and tobacco smoke. Potential biomarkers of exposure to 1,3-butadiene are two urinary mercapturic acids (monohydroxybutenyl-mercapturic acid (MHBMA) and dihydroxybutymercapturic acid (DHBMA)) as well as various haemoglobin and DNA adducts. Urinary MHBMA has turned out to be the most specific biomarker for quantifying the smoking-related exposure to 1,3-butadiene. We report here various liquid chromatography methods with tandem mass spectrometric detection (LC-MS/MS) for the determination of MHBMA and DHBMA in urine. Various pitfalls in the analysis of these biomarkers are also presented. Finally, data for MHBMA and DHBMA in urine of non-smokers and smokers measured in our laboratory and reported in the literature are presented. From the data available, we conclude that urinary MHBMA is presently the most suitable biomarker for smoking-related exposure to 1,3-butadiene.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 20

Determination of selected N-nitrosoamino acids in smokeless tobacco products

IARC Monograph 89 summarised the presence of 28 chemical agents in smokeless tobacco products (STPs) including a number of nitrosated species such as N-nitrosonornicotine (NNN), 4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and four N-nitrosoamino acids (N-nitrososarcosine (NSAR), 3-(N-methylnitrosamino) propionic acid (MNPA), 4-((N-methylnitrosamino) butyric acid (MNBA) and Nitrosoazetidine-4-carboxylic acid (NazCA)). Several researchers have also reported the presence of other N-nitrosoamino acids in tobacco. Over the last 20-30 years a significant number of studies have characterised the contents of NNN and NNK in STPs, and research has shown changes in levels of these compounds over this time period. However, the nitrosoamino acids have received significantly less attention. Given the lack of pertinent information in this area, a survey of the levels of 11 N-nitrosoamino acids was considered of interest to more fully characterise the chemistry of currently available STPs. The analytical methods used historically for the determination of N-nitrosoamino acids were mainly based on GC-TEA analysis, which required the derivatization of N-nitrosoamino acids and often required multiple extraction procedures in order to recover all of the relevant compounds. No method suitable for all eleven N-nitrosoamino acids has been published.

In this study an HPLC-MS/MS method was developed for the quantification of eleven N-nitrosoamino acids in a range of STPs. The compounds were extracted in water, trapped on a diatomaceous earth cartridge and separated on a polar HPLC column. Detection was performed by ESI-MS/MS. Quantification was achieved using a standard addition procedure, using deuterated analogues of three selected N-nitrosoamino acids as internal standards. In order to exclude artefactual formation of nitroso compounds during the sample preparation, a substance that does not occur naturally in tobacco was added at the beginning of the sample extraction and its nitroso analogue was monitored. The method was demonstrated to be fit for purpose for the quantification of the 11 N-nitrosoamino acids in various types of STPs.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 21

Determination of angelica lactones in smokeless tobacco products

IARC Monograph 89 summarised the presence of 28 chemical agents or carcinogens in smokeless tobacco products (STPs), including α- and ß-angelica lactones (Figure 1). However, no information is available in the literature on their concentrations in STPs, neither are there any available analytical methods for their determination in STPs.

A) alpha-angelica lactone

B) beta-angelica lactone

Figure 1. Structures of alpha-angelica (A) and beta-angelica (B) lactone.

In order to establish whether they are present in contemporary STPs an analytical method was developed for their analysis and quantification. The method is based on SPME extraction of the compounds from the tobacco matrix followed by HS-GC-MS analysis, using a WAX column and SIM detection. Quantification of α-angelica lactone is achieved using standard addition in combination with acetophenone-d_3 as the internal standard. The fitness for purpose of the method is demonstrated, e.g. the limit of quantification is 65 ng/g and the calibration range is 65-430 ng/g. The method for ß-angelica lactone is only semi-quantitative, as no reference substance could be obtained for this compound. Hence, quantification is achieved using α-angelica lactone as reference and assuming a similar response. During the work it was noted that degradation of angelica lactones during sample extraction may occur in protic solvents.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 05

Comparison of gene expression profiles of cigarette smoke-exposed normal human bronchial epithelial (NHBE) to profiles from smokers and patients with chronic obstructive pulmonary disease (COPD) and lung cancer

Molecular changes in multiple signaling pathways that regulate oxidative stress, inflammation and xenobiotic metabolism are frequently observed in diseased lung. The purposes of this study were: 1) to assess differential gene expression profiles in human lung tissue or cells of non-smokers and smokers with and without lung cancer or COPD and 2) to compare gene expression changes induced by smoke in *in vitro* cultures of NHBE cells to expression profiles from *ex vivo* lung tissue samples.

Gene expression profiles were evaluated with the Affymetrix HG_U133 Plus 2.0 GeneChip Microarray. First, expression profiles which distinguished normal tissues from non-smokers and smokers were represented by 115 statistically significant probe sets (genes). Pathway and gene ontology analyses identified several ontologies containing overrepresented genes in smokers, including those involved in immune response and T cell activation. Second, gene profiles (7880 probe sets) and molecular pathways that distinguished malignant lung of smokers from the paired normal tissue were associated with immune response, cell cycle regulation and tryptophan metabolism. Third, comparisons of bronchial brushings from non-smoker, smoker and COPD (GOLD 0 - II) yielded 63 unique probe sets. NADPH quinone oxidoreductase (NQO1), mucin 5AC and glutamate decarboxylase 1 were among notable targets, and the top pathways represented oxidative stress, xenobiotic metabolism and hypoxia.

Finally, as we previously reported, the top responders in the NHBE cells exposed to repeated applications of cigarette smoke condensate (CSC) were represented by 241 consistently regulated probe sets with significant deregulation of genes involved in oxidative stress, xenobiotic and tryptophan metabolism. Bioinformatic analyses were further employed to identify correlations with smoke and disease-impact in *ex vivo* lung samples. The gene profiles that were significantly modified in both CSC-exposed NHBE cells and COPD lung brushings included aldo-keto reductases (AKR1B10, AKR1C1, AKR1C2), cytochrome P450 1B1 (CYP1B1), NQO1, glutathione peroxidase 2 (GPX2) and SLC7A11, an amino acid transporter. Probe sets significantly regulated in both CSC-exposed NHBE cells and the cancerous and smoking-exposed lung were osteopontin (OPN/Spp1), dedicator of cytokinesis 10 (DOCK10), glycerol kinase (GK), kynureninase (KYNU) and transforming growth factor beta 2 (TGFB2).

Collectively, the data suggest that smoke-induced expression profiles in *in vitro* lung cell models are phenotypically relevant to lung modifications *in vivo* and may serve as potential biomarkers of effect.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 06

Evaluation of cigarette smoke-induced cell transformation using three *in vitro* cell transformation assays

Cell transformation assays (CTA) detect phenotypic changes associated with neoplastic transformation and available evidence suggests *in vitro* cell transformation may stimulate early components of the multistage process of carcinogenesis. Moreover, CTAs may aid in identifying potentially non-genotoxic carcinogens that may not be detected by conventional genotoxicity assays. We have tested whether total particulate matter (TPM) and cigarette smoke condensate (CSC) generated from Kentucky reference 3R4F cigarettes induce morphological transformation using three versions of the CTA: the Syrian Hamster Embryo (SHE) cell transformation assay using a 24-hour and 7-day exposure protocol, the SHE assay using an initiator/promotor protocol, and an initiator/promotor assay using B-has 42 cells (*v-Ha-ras*-transfected Balb/c 3T3 cells). Both TPM and CSC were positive in the SHE 7-day exposure with 12.5- and 9-fold increases, respectively, in the number of morphologically transformed colonies as compared to solvent controls; CSC was positive (2.7-fold increase) while TPM was equivocal in the SHE 24-hour exposure assay. TPM and CSC each induced positive responses in the SHE initiator and promotor assays with approximately 3-fold increases in the number of morphologically transformed colonies. In the Bhas-42 initiator assay, TPM produced a positive response (3.5-fold increase) while CSC was equivocal; however, in the B-has 42 promotor assay both TPM and CSC were positive, inducing approximately a 12-fold increase in morphologically transformed foci. Among the CTA models investigated, the B-has 42 promotor assay provided the most linear responses coupled with a robust increase in transformed foci across the treatment range and may therefore be a useful technique to evaluate cigarette samples.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 22

Mouth level exposure time on nicotine and TSNA extraction from snus pouches

Measuring the amount of constituents extracted by consumers of snus during use is a valuable step in estimating exposure of snus users to tobacco constituents. A factor potentially influencing the extent of exposure is the length of time that individual consumers keep snus pouches in their mouths during use. A recent survey of snus use in Sweden established that, on average, use of pouched snus extends from approximately 30 minutes to just less than 2 hours. The objective of this study therefore was to quantify the importance of use duration on constituent transfer from snus to the consumer over this time-scale.

30 volunteer pouched snus users took part in a central location trial in Sweden, each using portions of snus over ten different duration periods (from 5 to 120 minutes), randomly ordered over 3 sessions. Used and unused portions were analysed for nicotine and tobacco-specific nitrosamines; the differences in measured quantities provided an absolute and % measure of transfer from pouch to consumer during use.

Transfer of nicotine and TSNAs was found to increase with increasing usage duration, with a mean transfer of 5% after 5 minutes use increasing to about 50% after 120 minutes use. Regression analysis confirmed a statistically significant ($p<0.001$) relationship between percentage transfer and usage duration. Analysis indicated the general operation of first order kinetics; although concentration-gradient driven Fickian diffusional processes were also supported. The observations are also consistent with recent pharmacokinetic studies (e.g. Digard *et al*., SRNT 2011, Lunell & Lunell, 2004), which showed that t_{max} for peak plasma nicotine concentration also appears to be linked to duration of snus use.

Exposure of snus users to tobacco constituents such as nicotine and TSNAs increases significantly with the duration of use.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 42

Risk reduction – impact of burn additive types on the diffusivity level of cigarette paper

It is known that cigarette paper diffusivity has an impact on the mainstream yields of cigarettes; particularly on the CO and the gas phase yields. Increasing the diffusivity level of cigarette paper significantly reduces the mainstream CO yield.

Burn additives are usually added to cigarette paper in order to improve the ash appearance and modify the combustion characteristics; thus having an influence on mainstream yields. Application of burn additives has no effect on the diffusivity of cigarette paper when these measurements are made on cigarette paper at room temperature. Heat treatment of the cigarette paper at 230 °C for 30 minutes and subsequent measurement of the diffusivity illustrates that the burn additive type and level have significant effects on the cigarette paper diffusivity.

In the course of the study it was found that, by using bicarbonate salts as burn additives, the heat treated paper diffusivity level can be increased, when compared to cigarette papers impregnated with citrate salts; which are the most common types of burn additives used in cigarette paper.

The experimental design involved treating cigarette papers with different loading levels of potassium bicarbonate ($KHCO_3$) and tri-potassium citrate (K_3Cit). Concentrations in the range of 1-6% were applied. For comparative reasons, the amounts of both burn additives have been calculated to the same molar concentration as potassium.

A comparison concerning diffusivity and other physical parameters of the cigarette paper samples produced with the respective burn additives will be presented.

Cigarettes have been produced by using cigarette paper, containing both burn additives at different concentrations. Mainstream smoke data from these cigarette samples will also be presented.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 40

Research on relationship of thermal conductivity, thermal diffusivity, volume thermal capacity of cut tobacco with temperature

The thermal properties of cut tobacco, such as thermal conductivity, thermal diffusivity and volume thermal capacity, are essential to the analysis of heat and mass transfer in various conditions of tobacco processing. The thermal properties of cut tobacco are influenced significantly by processing temperature. In this work, the experimental equipment was established for measuring the thermal properties of cut tobacco under different temperatures. Moreover, the repeatabilities and systematic errors of experimental data were investigated with a transient plane thermal source method at different measuring levels, and the relationship between thermal properties of cut tobacco and temperature were studied. The experimental results are as follows: (1) within the measured temperature range, the change rule of thermal properties of cut tobacco differed at different stages; (2) the standard deviations of repeatability of test results of thermal conductivity, thermal diffusivity and volume thermal capacity of cut tobacco were 0.00583 $Wm^{-1}K^{-1}$, 0.02028 mm^2s^{-1} and 0.16316 $MJm^{-3}K^{-1}$, respectively; (3) thermal conductivity of cut tobacco significantly linearly correlated to temperature at the first change stage.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 07

Evaluation of the diagnostic accuracy of a candidate biomarker to detect use of conventional cigarettes in users of tobacco products that do not burn tobacco

Objective: In clinical studies to assess alternative tobacco products that heat rather than burn tobacco, a compliance biomarker is needed to detect the additional use of conventional cigarettes. Standard biomarkers of exposure to cigarette smoke constituents seem to be not sensitive enough for this purpose. The aim of this research was to identify and characterize a biomarker specific for combusted tobacco.

Methods: 2-cyanoethyl mercapturic acid (CEMA), a metabolite of acrylonitrile, was identified as a candidate compliance biomarker. CEMA was analyzed in 24-hour urine samples retained from a clinical study (#NCT00812279) that assessed a test product that heats rather than burns tobacco. The diagnostic accuracy of three different CEMA analysis variables (concentration [$CEMA_c$], amount excreted in 24 hours [$CEMA_{Ae24h}$], CEMA concentration normalized by urinary creatinine [$CEMA_{creat}$]) was evaluated by analyzing the receiver operating characteristics (ROC) curves. The diagnostic accuracy of CEMA variables and standard biomarkers of exposure was compared based on the area under the ROC curves.

Results: $CEMA_{creat}$ and $CEMA_c$ were able to differentiate between current smokers of conventional cigarettes and both non-smokers and smokers of the test product with high diagnostic accuracy. The performance of $CEMA_{creat}$ was slightly better than that of $CEMA_c$. Both CEMA variables performed better than standard biomarkers of exposure.

Conclusion: The results suggest that $CEMA_{creat}$ is a suitable biomarker for assessing product compliance in clinical studies assessing alternative tobacco products and allow recommendation of preliminary thresholds to detect recent use of conventional cigarettes based on $CEMA_{creat}$ levels. This will be verified in future clinical studies.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 24

Number of laboratories in machine smoking studies – some simulation experiments

One of the main objectives of a ring trial is the determination of repeatability and reproducibility variances for the population of the participating laboratories. In machine smoking studies a broad spectrum of influencing factors on the test results is inherent to the measurement procedure and therefore a certain number of laboratories is necessary to estimate the mean level and the variability measures of the analyzed variable with a certain trueness and precision.

This study investigates the effects of the number of laboratories on the grand mean and the variability measures. Several simulation studies have been performed on the basis of large studies like the EUCS Studies (European Collaborative Studies) and the Collaborative Study 2010 within WG10 of ISO-TC126. In a study performed for example by 3 laboratories only the grand mean would be clearly over- or underestimated in nearly 50% of all possible study combinations. Even more extreme is the situation when the variability measures are considered. The simulation studies will give some rough ideas about acceptable number of laboratories for this type of study.

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Explanation of outlier analysis - remarks based on the evaluation of the WG10 study

In 2010 a collaborative study was undertaken to investigate the intra- and inter-laboratory variability of the determination of mainstream smoke NFDPM, nicotine and carbon monoxide yields from a range of commercial cigarettes, a reference cigarette and the CORESTA CM6 Monitor Test Piece. These test articles were smoked in accordance with ISO and Health Canada Intense (HCI) methods (the study with its main results is described in the presentation of D. Mariner *et al*.). In the statistical analysis of this study we proceeded according to ISO 5725 in general. Concerning the outlier analyses different strategies can be applied for this large and structured data set. These strategies will be explained and described in their effects on final estimates like the intra- and inter-laboratory variability. In addition to the "standard" analysis of a data set statistical simulation experiments will underline possible effects of outlier analysis when certain data structures are given (e.g. bimodal distributions).

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 49

A 24 factorial experimental design to investigate the influence of LIP testing substrate parameters (basis weight, permeability and roughness) on LIP pass rates and residual length measurements

Lower Ignition Propensity (LIP) cigarettes have modified cigarette paper that aims to demonstrate lowered ignition propensity under standard laboratory tests (ASTM E2187-09:2009 and ISO 12863:2010). These cigarettes must meet performance criteria that are determined by regulations, in which a fixed percentage of lit cigarettes must self-extinguish under controlled laboratory conditions.

The LIP test methods have an inherent variability; the sources of which may be related to the natural variability of cigarettes, the operator(s) or equipment used. In addition, it was thought that the manufacturing variability in several of the physical parameters of the substrate upon which the LIP test is conducted may have an influence on the LIP test variability. The aim of this study was to investigate how changes in three substrate paper physical parameters influence LIP pass rates, with the aim of understanding the degree to which the manufacturing variability of the substrate influences the LIP test.

In order to investigate the above, a 2^4 factorial matrix of nine substrate papers was manufactured by delfortgroup with basis weight (92 - 102 gsm) air permeability (180 - 260 CU) and roughness (1000 - 2000 ml min^{-1}) specifications. The LIP pass rates and residual lengths of two cigarette types (with differing LIP pass rates) were measured according to the ASTM E2187-04 test method by Arista Laboratories UK. Five replicates of LIP testing (40 cigarettes per replicate) were conducted in each case and the substrate papers were placed with their rough side facing upwards.

Multiple regression analyses of the LIP pass rate and residual length measurements were performed in Minitab 16. The results showed that cigarette type had the largest influence on LIP pass rates and residual length measurements. Of the three substrate physical parameters studied, roughness had the most significant influence on LIP pass rates and residual lengths, whereby increasing the roughness significantly decreased LIP pass rates and residual lengths. Increasing the substrate basis weight was found to increase LIP pass rates.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 36

Matrix effects on analysis of multi-pesticide residues in tobacco by LC-MS/MS and GC-MS

A fast and easy multi-pesticide residue analyzing method for 73 and 19 pesticides in tobacco by LC-MS/MS and GC-MS respectively was introduced. The matrix effects and the deviations of detecting results were discussed as the main points of this thesis in several approaches. Firstly, the contributions of different tobacco types to matrix effects were compared, including burley, oriental and flue-cured tobacco samples. Secondly, the influences of dilution on matrix effects were shown as different detecting responses along with dilution times, which illustrated that proper dilution could minimize matrix effects, although not completely. Thirdly, the moisture content of the sample was regarded as one of the factors affecting extraction efficiency and matrix effects. The addition of moisture to the dried sample benefited the extraction and minimized matrix effects. However matrix effects could not be minimized only by using an internal standard because the matrix affected each analyte differently. Therefore matrix-matched standard solutions were necessary. By investigating the influencing factors of matrix effects and how to minimize them effectively, the detection results of the multi-pesticide residue analyzing method were improved.

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Generalized additive models for analyzing non-linear relationships between conventional chemical compositions and smoking quality of flue-cured tobacco

Based on the generalized additive model, an identification method of non-linear relationship was constructed. The linear and non-linear relationships between the conventional chemical components (such as total nitrogen, nicotine, total sugar and reducing sugar, etc.) and smoking quality of flue-cured tobacco were studied with 497 middle flue-cured tobacco leaf samples from the main tobacco producing areas in China. The results showed that: 1) at the 0.05 level, total nitrogen significantly negatively linearly correlated with aroma quality and quantity, offensive odor, irritancy and aftertaste; nicotine showed a significant negative linear correlation with aftertaste and a significant positive linear correlation with aroma quantity; total sugar and reducing sugar significantly positively linearly correlated with aroma quality and quantity, offensive odor, irritancy and aftertaste; 2) there were significantly non-linear relationships between total nitrogen and aroma quality, offensive odor, irritancy and aftertaste, between nicotine, total sugar, reducing sugar and aroma quality and quantity, offensive odor, irritancy and aftertaste, which showed an obvious "∩" shaped curve relationship; while the non-linear relationship between total nitrogen and aroma quantity was not significant at the 0.05 level; 3) the appropriate ranges of total nitrogen, nicotine, total sugar and reducing sugar contents were 1.5%-2.8%, 2.0%-3.5%, 15%-28%, and 15%-25%, respectively; 4) generalized additive model could consistently reproduce the original features of the curve and fully reveal the non-linear relationship between variables, which provided a new method for investigating the various forms of non-linear relationships between chemical composition and smoking quality of tobacco.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 17

Study of the pyrolysis of cigarette paper with added potassium salts

Potassium salts added in tobacco and cigarette paper can decrease tar and CO in mainstream smoke. In order to explore the effect of potassium salts in the pyrolysis of cigarette paper, cigarette papers with added potassium carbonate, potassium oxalate monohydrate, potassium citrate, potassium malate and potassium gluconate, respectively, were analyzed by Py-GC-MS, thermal analysis and determination of cigarette paper air permeability. Cigarette papers were pyrolysed at 300 °C, 500 °C, 700 °C and 900 °C in air atmosphere. The results showed that a) potassium salts could make cigarette paper pyrolyse at lower temperature; b) facilitate cigarette paper to pyrolyse completely, especially cigarette papers with added potassium carbonate, potassium oxalate and potassium citrate that made the key C-C and C-O bonds in the fiber rupture; and c) form micromolecules and rearrange into new substances at lower temperature. The result of thermal analysis showed that with the addition of potassium salts to cigarette paper, the formation of active cellulose was catalyzed violently and the higher the content of potassium, then the lower the cigarette paper initial weight-loss temperature. Also adding potassium salts to cigarette paper could increase its air permeability, and with the more potassium, the higher cigarette paper air permeability. The higher cigarette paper air permeability could reduce CO and tar in the mainstream smoke of cigarettes.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 48

Study of heat transfer of a cigarette relating to the ignition propensity

It is known that the cigarette ignition propensity, based on ASTM pass rate, is related to the cigarette mass burning rate (MBR) and heat output from the burning cigarette. However, there are few previous works to discuss the details in the cigarette ignition propensity from the view point of heat transfer. The objective of this study is to produce further understanding of the heat transfer of the burning cigarette on the substrate.

This study focuses on the influence of cigarette circumference in order to obtain the data of the cigarettes with various ignition propensities. The cigarettes with different circumference 17.0 mm, 24.9 mm and some blend types in the range of the MBR 30 mg/min ~ 60 mg/min were measured for the ASTM pass rate. Furthermore, the surface temperature of these cigarettes was measured using an infrared thermal-camera. Using these data and the Stefan-Boltzmann's equation, the heat output from the burning cigarette in contact with a substrate was estimated at local positions near the paper char-line.

It was shown that there was a difference in ASTM pass rate between cigarettes with the same MBR. The ASTM pass rate of the cigarette 17.0 mm in circumference was 5%, on the other hand, that of 24.9 mm was about 80%. Furthermore, the surface temperature in contact with a substrate proved to be higher than in the case of the smaller circumference. Also, the cigarette 17.0 mm in circumference had twice as large heat output at local position, that is from the cigarette combustion area (0 mm) to the cigarette char line (~4 mm), as that of 24.9 mm.

These results indicate that not only the understanding of the effect of MBR, but also that of heat transfer of the burning cigarette on the substrate are important for more accurately estimating the ignition propensity in various cigarettes.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 37

Studies on chemical changes of low polar components in fresh tobacco leaf

Several low polar components in fresh tobacco leaf are widely accepted as the precursors that decompose into their metabolites relating to the taste and aroma of cigarettes during the curing process. However, the metabolic pathways of some low polar types still remain unclear. This study thus aims to investigate the obscure metabolic pathways of chlorophyll and plastoquinone by chemically treating them. To this end, tetrahydrofuran extract from fresh tobacco leaf (Japanese domestic air-cured) was chemically treated under the following conditions: temperature - 30 to 100 °C; time - 6 to 100 hours; catalyst - formic acid and pyridine. A liquid chromatograph (LC) equipped with a photo diode array detector (PDA: 190-800 nm) and atmospheric pressure chemical ionization / mass spectrometer (APCI/MS: 100-1850 m/z) was selected for analysis, because it was considered useful for separating and simultaneously detecting chlorophyll, plastoquinone and their metabolites. The separation was performed under the following conditions: column - Excelpak SIL-C18 5C^{TM} (250 mm × 4.6 mm I.D); mobile phase A – acetonitrile; mobile phase B – acetone; flow rate - 1.0 mL/min; gradient condition - A 100% at 0 min, A 30% at 10 min, A 20% at 30 min, A 0% at 40 min, and A 0% holds until 55 min; column temperature – 25 °C. The generation of solanachromene (typical component in flue-cured Virginia) from plastoquinone was mainly observed in the extract heated at 70 °C with pyridine. This suggests that the electro cyclic reaction forming the chromenol skeleton of solanachromene required high temperatures, which is mainly applied to the flue-curing process. While formic acid smoothly converted chlorophyll into pheophytin, which subsequently decomposed into pyropheophytin with decarboxylation under high temperature, the generation of solanesyl pheophorbide (typical component in Oriental) was not detected regardless of the treating time, temperature and the type of catalyst. The outcome indicated that solanesylation of pheophorbide might occur through transesterfication or enzymatic reaction.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 26

Nicotine particle / gas phase distribution trends: denuder tube studies

Mainstream cigarette smoke comprises a complex dynamic mixture of many thousand substances resulting from the incomplete combustion of tobacco. Substances may be distributed within the gas and / or particle phases of smoke. A specific substance, such as nicotine, may also be distributed between both gaseous and particle phases. An advanced denuder tube design has been developed to study dynamic particle / gas phase equilibria, described previously, with emphasis on controlled smoking environmental parameters such as relative humidity (30, 45, 60% RH), flow rate (5 cm^3 s^{-1}) and denuder temperature (298, 310, 323 K). The experiments provide an estimate of gas phase nicotine levels in smoke from the amounts deposited on the denuder walls. The fraction of nicotine deposited on the walls is influenced both by smoking relative humidity and denuder tube temperature. As experimental temperature increases, the fraction of nicotine depositing on the walls increases. Increasing atmospheric RH was found to reduce the fraction of nicotine captured by the walls, particularly at the lower temperatures of the study.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 17

Can we continue to ignore chemical constituents of novel smokeless tobacco products other than the GothiaTek® analytes?

As part of our ongoing program at Lauterbach & Associates to characterize the chemical constituents of tobacco products other than cigarettes, we had begun in early 2010 to obtain samples of novel smokeless tobacco products that had been introduced on the US market over the past several years. After we had completed most of our analyses and submitted our abstract for presentation of our research at the 241st national meeting of the American Chemical Society (Paper #192 AGFD), we learned of the publication of similar research by Goodpaster and co-workers at IUPUI [J. Agric. Food Chem., **2011**, 59 (6), pp 2745-2751]. Comparison of our results with those Goodpaster's revealed several differences both in analytical approach and the conclusions reached from the analytical data and these differences will be the subject of this presentation. Our approach was to use the same GC-MS scan techniques that we had used for our previous studies on conventional smokeless tobacco products (STP) (SSPT11 CORESTA 2009). Goodpaster's approach was similar and also included SPME analysis of tobacco volatiles; however, his sample preparation and GC-MS analysis conditions did not provide the depth of analysis that our techniques did. Indeed, Goodpaster missed a compound that has apparently not been previously reported, and we found in our DS scans of several very different styles of novel STP. It may be a condensation product of an uronic acid and another monosaccharide. Retention time was about 102% of sucrose octa-TMS and typical *m/e* data were 73 (100), 308 (60), 307 (37), 309 (33), 343 (30), 310 (27), 129 (25), and 361 (22). In addition to discussing our GC-MS data, we will comment on other aspects of Goodpaster's article (calculation of the so-called free nicotine; incorrect interpretation of toxicological data) that have put erroneous information in the peer-reviewed literature.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 55

Metabonomics study on effects of sidestream cigarette smoke on urinary metabolites of rats

The endogenous and exogenous urinary metabolites of rats passively exposed to sidestream cigarette smoke (SCS) were analysed to study the changes of urinary metabolites with a metabonomics method. The contents of total urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), total 1-hydroxypyrene (1-HOP) and 3-hydroxybenzo[a]pyrene (3-HOBaP) in urine sampled from rats of three groups; a control group, a group treated with blended reference cigarettes (non-menthol) and a group treated with menthol cigarettes, were determined to assess the toxins of exposure to SCS. The total NNAL level was determined by LC-ESI-MS/MS and the levels of 1-HOP and 3-HOBaP were determined by ultra performance liquid chromatography (UPLC) with fluorescence detection (FLD). The urinary endogenous metabolites of all samples were analysed with UFLC / IT-TOF-MS, the data were processed with chemometrics, and the effects of exposure time were evaluated. Eleven endogenous metabolites related to smoke exposure were identified, their contents were compared, and the results showed that SCS had complex effects on rats. The contents of total NNAL and 3-HOBaP in samples from the menthol cigarette group were statistically significantly lower than those from the non-menthol cigarette group (P <0.01 and P <0.05, respectively), whereas 1-HOP content was statistically unchanged (P >0.05). The sidestream smoke of menthol and non-menthol cigarettes influenced methyl-hippuric acid, glucuronate, and pantothenic acid greatly, however xanthurenic acid was not affected. The contents of kynurenic acid and N2-succinyl-L-ornithine in urine of rats passively exposed to sidestream smoke of non-menthol cigarettes decreased, while suberylglycine content in that of menthol cigarettes increased significantly. Menthol in cigarettes had positive and negative effects on the amino acid metabolisation in rats. In general, the urinary metabolic profiling of the menthol cigarette group was closer to that of the control group.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 15

Development and validation of a UPLC-MS/MS method for quantification of mycotoxins in tobacco and smokeless tobacco products

Mycotoxins are a group of naturally occurring toxic/carcinogenic substances that could be found in a wide range of food commodities, but can also be found in tobacco and tobacco products. Many countries have adopted regulations to limit exposure from food. In Sweden, Aflatoxins are regulated in food and smokeless tobacco products (STPs), and Aflatoxin B1 is also included in the "Draft Proposed Initial List of Harmful/Potentially Harmful Constituents in Tobacco Products" presented by the FDA. Today there is no recommended method for the simultaneous analysis of Mycotoxins in tobacco and STPs. Therefore, a quantitative multi-method for determination of four Aflatoxins and Ochratoxin A has been developed and validated.

The samples are extracted with a methanol/water mixture and diluted with PBS buffer after the addition of deuterium-labelled internal standards. The aqueous extract is then transferred to a Gilson ASPEC^{TM} XL4 robot for automated clean-up using immunoaffinity columns. By using UPLC-MS/MS for quantification, instead of traditional HPLC-FLD, the analysis time is typically reduced by 75% to below a 4 min cycle time. The considerable reduction in analysis time by using this robot and UPLC-MS/MS system offers significant benefits to laboratories undertaking routine analysis of Mycotoxins in tobacco and tobacco products as well as in food products.

During the initial method development, one main problem was to achieve a homogeneous tobacco sample. Therefore, a procedure for the homogenization of the tobacco was developed in order to obtain accurate and reproducible results. This method showed high sensitivity with low quantifications limits of 0.1 µg/kg and 0.5 µg/kg for Aflatoxins and Ochratoxin A, respectively. The recovery rates were between 87 and 106% and the precision (CV) below 20% for all analytes. When participating in several proficiency tests for food items organised by FAPAS using this method our laboratories have obtained very good Z-scores.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 50

The influence of the dynamic smoke flow underneath perforated tipping paper on the sensory properties of cigarette smoke

Filter ventilation is one of the principal methods to control the smoke stream between the tipping and the filter plug wrap paper or between the plug wrap and the filter material respectively. Therefore, perforation of tipping paper is an essential tool to adjust the design of a cigarette for a proper generation of specific smoke deliveries. Besides the determination of stationary flow properties and smoke yields based on various smoking regimes, the investigation of the dynamic smoke flow underneath perforated tipping paper provides a profound knowledge about the impact of diluted cigarette smoke on the human sense of taste. Physical and geometrical parameters of individual perforation processes are the basis for a numerically derived dynamic smoke flow simulation model on the one hand and the influence on the cigarette taste on the other hand. In order to study the correlation between the aerosol distribution with a smoker's physiological perception of the cigarette taste, surveys amongst regular cigarette smokers and with a professional smoker panel were performed. The evaluations and experiences from these surveys reveal a practical confirmation for the proposed theoretical approach, and, moreover, the findings open the potential to apply different configurations of tipping paper perforation for adapting the sensory properties of cigarette smoke to targets requested by cigarette manufacturers and consumers.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 25

Chemical, physical and thermodynamic evaluation of arsenic species and their transformation during cigarette smoking

Tobacco smoke contains metals such as chromium (Cr), cadmium (Cd) and arsenic (As). The levels of Cr, Cd and As in mainstream cigarette smoke normally range from a few to a few tens of parts-per-million (ppm) in modern filtered cigarettes. The toxicity of these metals is specific to their chemical forms as well as their concentrations in cigarette smoke. It has also been noted in the literature that redox properties of these metal species are also an important factor to consider because of their potential to mediate free radical reactions in cigarette smoke. Very little speciation information exists for these metals either in tobacco samples or in cigarette smoke. Moreover, it is desirable to understand their transformation during the smoke formation processes under puffing and smouldering cycles. In this study, we combined analytical chemistry (HPLC-ICP-MS), synchrotron-based X-ray Absorption Near-Edge Structure Spectroscopy (XANES) and thermodynamic prediction to elucidate the transformation reactions of As species during the mainstream smoke formation process. XANES results revealed a dynamic transformation from As(V) in 3R4F tobacco leaf to As(III) species in fresh mainstream smoke, although the exact species involved in the reactions cannot be determined due to the detection limit of the technique. The direction of this redox reaction was reversed upon smoke ageing. Using HPLC-ICP-MS, percentages of the water-soluble As species in cut tobacco and the mainstream smoke have been identified and they included species such as methylarsonic acids (DMA, MMA) and arsenobetaine (AsB). In addition, the chemical analysis on fresh and aged smoke samples confirmed the As(III) and As(V) redox reaction, thus allowing for a consistent picture to be built for the dynamic transformation of As species during smoke formation.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 47

Influence of base paper citrate and filler amount and of band diffusion on smoke deliveries, ASTM and FASE

Having a tool that can predict both smoke deliveries and fire propensity test results has become necessary because of tobacco regulations, and acceptability to the consumer.

The work presented in this paper is a systematic study on the role of base paper and band parameters on smoke and on ASTM and FASE.

To conduct this study, we have used a face centred central composite design.

Because of its positive impact on CO deliveries, we have chosen to use K citrate as the burn additive.

For band geometry, we have used one of the most common design: 6 mm band width and 20 mm band spacing.

For the 3 levels of variations requested in this type of study we have used:

- Base paper amount of K citrate: 0.8 ; 1.4 and 2%
- Base paper amount of $CaCO_3$: 25 ; 29 and 33%
- Band diffusion: 0.055 ; 0.095 and 0.135 cm/s

Two blends were used in the study: American blend and Virginia.

The study led to a number of conclusions, which will be presented: the rules known for smoke deliveries, i.e. the impact of filler level and citrate, mainly on CO, apply with the banded papers.

Band diffusion, as expected, shows a major impact on the cigarettes extinction.

But what we also found is that the amount of K citrate has a strong and major influence on ASTM and FASE, as filler level but to a lower extent.

Detailed results will be shown, showing that the design of LIP cigarette papers can be optimised depending on constraints for tar, CO, puff number and ASTM, FASE requirements.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 19

The influence of nitrogen oxides on determination of hydrogen cyanide in mainstream cigarette smoke by continuous flow analyzer and the improvement of the method

Hydrogen cyanide has been well known as a possible source of toxicant in cigarette smoke. Accurate determination of hydrogen cyanide is a great help to accessing the risk of cigarette to public health. In the conventional methods for determination of hydrogen cyanide mainstream cigarette smoke, alkaline solution was used to collect hydrogen cyanide. In our study, nitrogen oxides in the smoke were found to dissolve in the alkaline solution, which could react with alkaline solution to produce nitrates and nitrites that can further react with cyanide to result in the underestimation of the yield of hydrogen cyanide. An improved method for the determination of hydrogen cyanide was developed in our laboratory to solve the problem, in which the hydrogen cyanide from cigarette mainstream smoke was collected using a Cambridge filter treated by ethanol and water solution of sodium hydroxide and detected by continuous flow analyzer based on a coloring system with isonicotinic acid and 1,3-dimethyl barbituric acid. The collection efficiency of hydrogen cyanide for this method was significantly improved compared to conventional methods trapping hydrogen cyanide using alkaline solution. Besides, the collection and sample preparation process was simpler with higher stability of the collected hydrogen cyanide. Limit of detection for hydrogen cyanide was less than 1.21×10^{-2} mg/L. The method achieved excellent reproducibility: the relative standard deviation for inter assay was 4.44% and the RSD for intra assay was 3.58%. The recoveries and the accuracies were in the range of 99.13% to 100.37% and 92.39% to 99.01%, respectively. This method is suitable for routine analysis of hydrogen cyanide in cigarette mainstream smoke accurately.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 44

Effects of pulp composition of cigarette papers on the characteristics of cigarette smoke

The pulp composition of cigarette papers was considered to evaluate the effects of flax and wood fibers of cigarette papers on the characteristics of cigarettes. In the cigarette system, the partial and total replacement by flax and wood pulp cigarette papers was carried out to simulate the effects of flax and wood fibers. The regular and ultra-slim cigarettes with flax pulp cigarette papers were replaced by wood pulp cigarette papers and the tobacco column was filled with flax and wood cigarette papers instead of cut tobacco leaves, respectively.

The mainstream smoke, whole smoke components and similarity of smoke were analyzed. It was concluded that for this particular tobacco blend and tar level, the partial and total replacement of flax paper and tobacco with wood paper resulted in similar levels of mainstream and whole smoke components.

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The 2010 ISO TC126 Working Group 10 Collaborative Study on intense machine smoking

ISO Technical Committee (TC) 126 Working Group (WG) 10 was established in 2007 by TC126 to undertake preliminary work in preparation for the standardisation of an intense machine smoking method. Recognising the impact of decisions taken by the Conference of Parties (COP) to the WHO Framework Convention on Tobacco Control, WG10 was required to take into account any relevant method proposals made by the COP. In 2010 a collaborative study was undertaken to investigate the intra- and inter-laboratory variability of the determination of mainstream smoke NFDPM, nicotine and carbon monoxide yields from a range of commercial cigarettes, a reference cigarette and the CORESTA CM6 Monitor Test Piece. These test articles were smoked in accordance with ISO and Health Canada Intense (HCI) methods. 35 Laboratories from 21 countries reported data with six laboratories reporting data from two smoking machines. Intra-laboratory variation was lower for HCI than ISO for all parameters. Inter-laboratory variation for HCI was higher than ISO for TPM, water and NFDPM, but not CO, along with the measurement uncertainty for all parameters. The water yields were disproportionately higher than other measured parameters under the HCI regime. Smoking machine type was the major contributor to the inter-laboratory variation under the HCI regime with higher values for TPM, nicotine, water and NFDPM and lower values for CO reported for linear smoking machines than rotary smoking machines.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 46

The impact of different physical and chemical cigarette paper base sheet parameters on smoke yields, and testing of an alternative substrate for Whatman #2 using the ASTM Method E.2187-09

Three cigarette paper parameters have been varied and their impacts on smoke yields have been measured. The basis weight has been kept constant at a level of 25 gsm, the chalk content at a level of 26%. All the samples are wood pulp based.

The following parameters were modified:

- the content of Trisodium and Trinatrium citrate (Mix) in the range of 0.75, 1.5, 2.25%
- air permeability in the range of 50, 100, 120 CU (ml/min/cm^2)
- diffusivity in the range of 0.100, 0.200, 0.300 cm/s

All cigarettes have been produced maintaining one specification with the same tobacco blend (American blend) to avoid interaction of other parameters.

All cigarettes were also tested for self-extinguishing rates according to ASTM E2187-09. ISO 12863 the international equivalent to the ASTM standard allows for the use of alternative substrates like the LIP CAN paper, a grade produced by the Finnish paper mill Tervakoski. All cigarettes have been tested on both substrate papers, Whatman #2 and LIP CAN and on three, ten and fifteen layers to achieve a better characterisation and discrimination of the self-extinguishing performance.

The differences in the self-extinguishing results were mainly caused by the changes in diffusivity.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 23

What you always wanted to know about smoking-associated oxidative stress, but did not dare to ask

The pathogenesis of numerous diseases related to cigarette smoking is firmly associated in the literature with a development of an oxidative stress. However, the current knowledge on oxidative stress as universal generator of pathologies and the existing methodologies for the assessment of its status do not provide an unambiguous understanding of the mechanisms of the biological effects of cigarette smoke. The main interrelations between (i) cigarette smoking, (ii) developing oxidative stress in the human organism and (iii) the most prominent diseases, whose pathogenesis is thought to be associated with smoking, become evident from the analysis of the statistics of scientific and medical publications and their dynamics over several decades. In this context, our survey encompasses mutagenesis and carcinogenesis, development of cardiovascular and neurodegenerative diseases, digestive diseases, pulmonary diseases including chronic obstructive pulmonary disease, allergic phenomena, alteration of the immune status of smokers and hypoxia. The difficulties in elucidating the influence of cigarette smoking on oxidative stress derive from fundamental problems in assaying oxidative stress status as well as understanding the interplay of pro-oxidative and antioxidant reactants in smoke and in the human organism. These issues make up the subject matter of our current analysis. The latter comprises a retrospective view (since the 1960s) on studying the phenomenon of oxidative stress in the context of cigarette smoking, which constitutes a major challenge for the scientific and methodological strategy for prediction and reduction of the harm in the design of future tobacco products.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 18

Comparison of filtration effectiveness using different adsorption materials

Tobacco smoke is a complex, dynamic and reactive mixture containing an estimated 5000 chemicals. This toxic and carcinogenic mixture is probably the most significant source of toxic chemical exposure and chemically mediated disease in humans. Therefore it is important to reduce these harmful compounds from tobacco smoke.

Selective filtration is a potential method to reduce toxic chemicals and triple filters are able to use various filtration agents such as charcoal, sepiolites and other natural or synthetic materials. The advantage of triple filter is that the additive is locked inside the filter by acetate segments to avoid fall out and contamination of the production line.

This study compared filtration effectiveness of several different commercially available additives. All tested filters were produced with the same specification and selected by the same weight. The filtration effectiveness was compared with triple acetate and monoacetate filters. Cigarettes with modified filters were hand made using CM6 tobacco columns. The cigarettes were weighed and smoked using the ISO smoking regime on Cambridge filter pads to collect tar.

The results of different tests of cigarettes made of the above mentioned filters show that applied filter adsorbents have different effects on tar removal.

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An induction process for 8-hydroxy-2'deoxyguanosine (8-OHdG) induced by aqueous extracts of mainstream cigarette smoke in cultured cells

Oxidative stress is a state of imbalance between the reactive oxygen species (ROS) and the ability of the organism to defend against them, leading to progressive oxidative damage. 8-hydroxy-2'deoxyguanosine (8-OHdG) is one of the markers for oxidative stress. Our previous results suggested that acrolein was a major factor of 8-OHdG induction in human alveolar epithelial cell line A549 treated with whole smoke-bubbled phosphate buffered saline (WS-PBS). In the current study, the formation process of 8-OHdG in A549 cells treated with WS-PBS was investigated, using a reference cigarette 3R4F. A decrease in intracellular glutathione concentrations was observed immediately after treating the cells with WS-PBS. When A549 cells were exposed to acrolein in an amount equivalent to that in the WS-PS treatment, of 3R4F, intracellular glutathione concentrations followed the same pattern as in WS-PBS. Formaldehyde and crotonaldehyde, which are known to interact with glutathione, did not contribute to the intracellular glutathione concentrations in the case of treating with an amount equivalent to these compounds in WS-PBS. Intracellular levels of hydrogen peroxide in cells treated with WS-PBS showed an upward trend until 30 minutes, after which they steadily decreased. In conclusion, it is speculated that 8-OHdG induction in cultured cells by WS-PBS progresses through the following steps: (1) decreased concentration of intracellular glutathione caused by the acrolein in WS-PBS; (2) a proportional increase of hydrogen peroxide in the cultured cells caused by a lowering of the cell's antioxidant ability as associated with the depleted intracellular glutathione levels; (3) hydroxyl radical induction from hydrogen peroxide by the Fenton reaction; and (4) oxidative damage to intracellular DNA by hydroxyl radicals induced from hydrogen peroxide.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 04

Analytical methodology for the determination of carbonyls in mainstream cigarette smoke - as an example for an appropriate method validation

The increasing demand from regulatory bodies regarding characterisation of tobacco and tobacco smoke products results in the need to develop and use methods, which are not available standard methods. These non-standardised methods have to be validated appropriately before being used in routine analysis. ISO standard 17025 required a comprehensive validation, which confirms that the method is fit for the intended use. However, the ISO standard 17025 also advises that the validation shall be as extensive as necessary to meet the needs of the given application.

Carbonyls as group of compounds from the Hoffmann list seem to be of major interest in the regulatory debates and therefore, a method validation for the determination of carbonyls in mainstream cigarette smoke was performed according to international guidelines. This group of compounds encompasses substances, such as formaldehyde, acetone, acrolein, acetaldehyde, propionaldehyde, n-butyraldehyde, crotonaldehyde and 2-butanone.

The cigarette samples selected for this study covered a range of ultra low tar products (ISO smoking regime) up to 36 mg NFDPM per cigarette (Canadian intense smoking regime). The regression coefficients of the applied calibration curves for each compound were calculated better than 0.995.

In this presentation, a typical experimental set up for a validation process and necessary validation parameters, e.g. precision, repeatability, recovery will be discussed. The confirmation of the accuracy and the reproducibility of the results were obtained by a collaborative study.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. IG 02

Agrochemical Analysis Sub-Group Report

The CORESTA Agrochemical Analysis Sub-Group is part of the Product Technology Study Group.

The Sub-Group's objectives are:

1) To perform regular proficiency testing of Multi-Residue Methods for the analysis of agrochemical residues on tobacco.
2) Undertake joint experiments to resolve unanswered questions arising from proficiency tests.
3) Produce and maintain a series of Technical Notes (on different agrochemical residue classes and selected individual compounds) to supplement the CORESTA Guideline N° 5 (Technical Guideline for Pesticide Residues Analysis on Tobacco and Tobacco Products) and aid in method development and improvement.

This paper will report on the Sub-Group activities in 2010 focusing on the outcome of the annual proficiency test, conducted in collaboration with FAPAS (part of the Food and Environment Research Agency, York, UK), the results of the Joint Experiment on Cyfluthrin and the degradation study on 8 agrochemicals. It will also present the various Technical Notes produced by the Sub-Group.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 35

Cigarette smoke toxicant exposure estimates in smokers switched to reduced toxicant prototype cigarettes

We have conducted a study that addresses the first part of the IOM's definition of a potential reduced-exposure product by analyzing biomarkers of exposure (BoE) in smokers switched to prototype cigarettes which, when measured on smoking machines, produced lower levels of some toxicants compared to conventional cigarettes.

The 6-week study, undertaken in Germany, involved short periods of clinical confinement. One hundred smokers of 6 mg ISO tar cigarettes were recruited. After 2 weeks smoking a commercial 6 mg ISO tar yield product, 50 were switched to a 6 mg ISO tar Reduced Toxicant Prototype (RTP) cigarette containing diluent sheet in the blend. In addition, 150 smokers of 1 mg ISO tar commercial cigarettes were recruited, 50 of whom were switched to an RTP containing the diluent sheet and 50 to an RTP containing tobacco treated to reduce polyphenols and proteins. All RTP cigarettes included filters containing adsorbant additives.

BoE, mouth level exposure to tar and nicotine were measured, and the sensory response to the product scored. Reductions in toxicant levels found in smoke chemistry correlated well with reductions in BoE. For example, the calculated average acrolein intake for the 6 mg ISO tar conventional product, based on cigarette yield under an intense regime and measured daily cigarette consumption was 2298 µg/day. This corresponded to an average level of 1803 µg/day of 3-hydroxypropylmercapturic acid (3-HPMA), a biomarker of exposure for acrolein. Similarly, the 6 mg ISO RTP was associated with a calculated acrolein intake of 871 µg/day and a 3-HPMA level of 751 µg/day. This equates to an approximately 40% reduction in both the estimated intake and the biomarker of exposure level.

Overall, the study also found that the groups switched to RTPs at both tar yields showed reduced levels of biomarkers of exposure for several smoke toxicants compared to those that continued to smoke conventional cigarettes.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 13

Rapid method for residual benzo[a]pyrene in tobacco and tobacco products by high performance liquid chromatography with fluorescence detector

Benzo[a]pyrene (B[a]P) is a polycyclic aromatic hydrocarbon, which is a by-product of incomplete combustion or burning of organic compounds. B[a]P is mutagenic and carcinogenic in nature. B[a]P is an ubiquitous compound both outdoors and indoors due to releases from multiple processes like vehicle emissions, burning of wood or coal in heating stoves or fire places. In tobacco it is formed during the curing process. Currently a method for the determination of B[a]P in tobacco is prescribed by Health Canada. It involves tedious multi-step sample cleaning with the use of a solid phase extraction cartridge (SPE) to remove matrix interference.

A quick and simple HPLC method is developed for the qualitative and quantitative determination of B[a]P residues in tobacco and snus without the use of the SPE cartridge. This method uses HPLC with a fluorescence detector. B[a]P is extracted from tobacco after refluxing with ethanolic KOH and liquid - liquid partition with a mixture of cyclohexane and chloroform and with distilled water. The cyclohexane extract is concentrated using a rota-evaporator and diluting with acetonitrile and subsequent chromatographic separation on LiChrospher RP-18e (250 mm × 5 micron × 4.0 mm) (Merck), under gradient condition using Mobile phase A- {Acetonitrile (70%), 1% iso-propyl alcohol in distilled water (30%)} and Mobile phase B- {Acetonitrile (100%)}.

The separations are monitored by fluorescence absorbance excitation at 365 nm and emission at 425 nm. The retention time for B[a]P is 14.2 ±0.5 min at a flow rate of 1.0 mL/min. The method has been validated by standard validation protocols i.e. limit of detection, limit of quantification, recovery, repeatability and reproducibility. Minimum recovery of 83% was obtained with a linear regression coefficient of 0.9999 for the range of 0.07-9.20 ppb of B[a]P. The limit of detection was 0.07 ppb. Tobacco samples of various grades and snus samples were analyzed.

This method provides several advantages such as sensitivity, speed, simple and easy sample preparation. The method has been found suitable for rapid determination of B[a]P in tobacco.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 14

Determination of propylene glycol, glycerol and sorbitol in tobacco and tobacco products by high performance liquid chromatography with refractive index detector

Propylene glycol (PG), glycerol and sorbitol are polyhydric alcohols and are added to tobacco as humectants / conditioners. PG acts as a solubilizing agent for flavors and also it hinders mold growth formation in tobacco. Glycerol and sorbitol are sweet in taste and are compatible with various ingredients used in tobacco mixture.

Currently a standard method for the determination of PG, glycerol and sorbitol in tobacco is CORESTA Recommended Method No 61. It involves the use of a polymer column that is costly, the life of the column is short when used at higher temperatures, and the mobile phase is a complex calcium salt of EDTA.

A simple HPLC method is developed for the quantitative determination of PG, glycerol and sorbitol in tobacco and snus samples with the use of an amino column and simple mobile phase of acetonitrile and water.

This method using HPLC with refractive index (RI) detector, involves the extraction of humectants from a sample of tobacco by an 80:20 mixture of acetonitrile and water and subsequent chromatographic separation on Intersil - amino (NH_2) column (4.6 × 250 mm × 5 µm) using an isocratic system, mobile phase - {acetonitrile : water (80:20)}. The separations are monitored by refractive index of each analyte. Retention time of PG, glycerol, and sorbitol are 4.5 min, 6.0 min, and 10.8 ±0.5 min at a flow rate of 1.0 ml/min.

Various parameters, i.e. composition of mobile phase, weight of sample and extracting solvent were optimized. Quantification is based on an external standard technique. The method has been validated by standard validation protocols, i.e. limit of detection, limit of quantification, recovery, repeatability and reproducibility. Minimum recovery of 92%, 94%, and 86% for PG, glycerol and sorbitol respectively were obtained with a linear regression coefficient of 0.9993, 0.9999 and 0.9993 for the range of 25 - 100 ppm of PG, glycerol and sorbitol respectively. The limits of detection of PG, glycerol and sorbitol were 25 ppm. Tobacco samples of various grades and snus samples were analyzed.

This method provides several advantages such as sensitivity, simple sample preparation and cost saving. This method has been found suitable for rapid determination of humectants for a large number of tobacco samples.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 56

Advanced filtration platform for cigarette smoke

The World Health Organization (WHO) Framework Convention on Tobacco Control (FCTC) has issued a recommended strategy for regulating toxicants in mainstream smoke (emissions). The regulation strategy specifies that the toxicants will be normalized by the cigarette delivered nicotine using the Canadian Intense smoking protocol. Toxicant reduction by filtration is a challenge due to the reduced filter resident time under the Canadian Intense protocol. Current carbon-on-tow and cavity filters technologies are first generation options, but mainstream smoke can by-pass the active particles and reduce their efficiencies.

A new filtration platform has been developed to address deficiencies of current carbon based filter technology (smoke by-pass). This filtration platform is based on a porous structure which eliminates smoke by-pass and channeling. The structure can achieve active particle loadings of 20 mg/mm and 8 mg/mm in standard and super slim filters respectively. As a result, significant toxicant reductions can be achieved under the Canadian Intense smoking protocol. Additionally, the porous structure is designed to match the pressure drop of a cellulose acetate filter.

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Per puff analysis of selective removal in ultra-slim filters

Understanding selective filtration is important for cigarette filter manufacturers to optimize cigarette filter performance to meet potential regulatory requirements. The analysis of selective filtration on a per puff basis provides insight into the changes in smoke chemistry and other factors which impact selective filtration mechanisms. Phenolic compounds are frequently used for selective filtration studies because these compounds are known to be selectively removed by cellulose acetate filters.

For this study, selective filtration of hydroquinone, phenol and other phenolic compounds were examined on a per puff basis for ultra slim and conventional filters. These compounds were determined by HPLC with fluorescence detection after extraction of Cambridge pads or filters in an alcohol solution. Direct analysis of extracts enables the phenolic compounds to be determined from the same smoking session as used to determine nicotine free dry particulate matter (NFDPM), nicotine, and CO.

The results showed increases in NFDPM delivery on the last whole puffs when the vents were closed relative to vents open for both conventional and ultra slim cigarettes. Ultra slim cigarettes had a much larger increase in NFDPM than conventional cigarettes when smoked to the nearest whole puff relative to the butt mark. Also, the delivery of phenol significantly decreased on the last puffs for both ultra slim and conventional cigarettes. The phenol delivery for the ultra slim cigarettes decreased on earlier puffs relative to conventional cigarettes. These results are important to cigarette filter manufacturers in understanding the smoke chemistry on a per puff basis for both ultra slim and conventional cigarettes and how the selective removal in the filter can change during smoking. It is also important to understand the impact of testing protocol on selective removal by a filter on a per puff basis.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 15

Fine-cut tobacco: annual TNCO study using a monitor tobacco blend

ISO Standard 15592-3 has been developed to determine nicotine free dry particulate matter ('tar') and nicotine in mainstream smoke of fine-cut tobacco smoking articles (FCSAs). Regular use of a reference material is recommended to assess if the analytical process is in control.

A first ESTA Monitor Tobacco ('EMT') blend was manufactured in 2004, following a large collaborative study with commercially available products performed between 2004 and 2006 where the major aim was to investigate fundamental questions in relation to repeatability and reproducibility and subsequent yield tolerances.

Since then, ESTA (European Smoking Tobacco Association) has offered interested laboratories the opportunity to participate in regularly conducted collaborative studies on ISO fine-cut tobacco smoke analysis using an EMT blend together with wrappers as specified by Annex B of the ISO Standard.

The purpose of these studies is to initially establish 'tar' and nicotine yields of the respective EMT blend and to provide a regular overview on repeatability and reproducibility.

Since they are carried out on an annual basis, the studies offer participants a means of proving their competence in ISO smoke analysis of fine-cut tobacco as required by ISO 17025.

Currently the third EMT blend ('EMT03') is in use and has been studied since 2009. Although the measurement of carbon monoxide ('CO') in mainstream smoke of fine-cut tobacco is not part of ISO 15592-3, this parameter has been included into the annual ESTA study since 2007.

Organisation of the annual EMT studies is supported by the ESTA Products Working Group - Technical Task Force and conducted in a fully anonymous way. Statistical evaluation is done in accordance with ISO 5725. In addition to the study reports provided to each participant, ESTA organises a meeting after each study for laboratory managers to exchange experience and address technical points of relevance observed during the study.

Participation in the EMT studies varies between 12 and 19 and includes laboratories from the tobacco industry, governmental institutes as well as privately-based independent laboratories.

The poster shows results of the 2011 study and also provides a comparison of results to those obtained in previous studies. Furthermore, the poster describes practical experience made with the EMT.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 53

Preparation of hydrophilic quaternary ammonium functionalized porous material for selectively reducing hydrogen cyanide in cigarette smoke

A porous adsorbent material bearing quaternary ammonium basic groups was synthesized by suspension copolymerization and the corresponding functionalization and then modified by the load of strongly hydrophilic substances. The structure of obtained hydrophilic quaternary ammonium functionalized porous material (HQAFPM) was characterized by FT-IR, N element analysis and nitrogen adsorption at 77K (a Micromeritics TriStar 3020M analyser was used and the surface area and the adsorption average pore width (4V/A by BET) were calculated using the BET method). The results showed that the prepared material with porous structure and relatively high specific surface area had been successfully modified by quaternary ammonium and hydrophilic substances. The effects of different functional reagents, hydrophilic substances and their concentrations on the removal of HCN from cigarette smoke were investigated using a model reactor, the results indicated that HQAFPM could significantly reduce hydrogen cyanide (HCN), and the material functionalized by Girard's reagent and modified by polyvinyl alcohol in 1% solution possessed the highest HCN reduction rate (48.2%). The obtained material was applied in the dual filter of cigarettes, the deliveries of HCN and crotonaldehyde in mainstream cigarette smoke were selectively reduced by 27.5% and 19.3%, respectively, compared with reference cigarettes; while the deliveries of tar, nicotine, CO, B[a]P, NNK, NH_3 and phenol changed little. The hazard index of the cigarette reduced by 0.7, while its sensory quality was almost unchanged. It was indicated that HQAFPM was a promising filter additive for selectively reducing HCN in mainstream cigarette smoke.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 32

Comparison of cigarette smoke cytotoxicities using different assay systems, Neutral Red uptake assay and WST-1 assay

Cytotoxic assays are used as part of a test battery to assess potential risk of cigarette smoke. So far, cytotoxic assays have been established indicative of various measurement endpoints, such as cell proliferation inhibition, enzymatic inhibition and membrane damage. The Neutral Red uptake (NRU) assay based on endocytosis is recommended by the CORESTA *In vitro* Toxicity Task Force to assess the cytotoxicity of cigarette smoke. However, it is considered that a more reliable outcome can be obtained in the toxicity assessment by combining several test systems with different detection mechanisms since each *in vitro* test detects a particular endpoint impact.

This study aims to investigate the specificity in cytotoxic assays, which measure cell viability with different detection mechanisms. The evaluation results from the NRU assay were compared with those of the WST-1 assay based on mitochondrial dehydrogenase.

A diverse set of the test cigarettes made of single grade burley or flue-cured tobacco was prepared for the experiment. The cytotoxicities of total particulate matter (TPM) and gas vapour phase (GVP) produced from the cigarettes were investigated with the NRU and the WST-1 assays. In addition, some chemicals such as constituents in cigarette smoke and cytotoxic compounds, which affect specific endpoints, were subject to both evaluation results.

Consequently, both assays provided the same outcomes in terms of the rank order for the cytotoxicites of TPMs and GVPs from a variety of the cigarettes. Regarding the responses to the smoke constituents, acrolein, hydroquinone and catechol showed responses common in both assays. Moreover, inhibitors of mitochondrial respiratory chain and chemicals causing membrane disruption also showed equivalent responses regardless of the assays based on different detection mechanisms.

Thus, it was suggested that the NRU and the WST-1 assays provided the same outcomes, and the difference in their detection mechanisms was not reflected in the assay result.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 20

An ion chromatographic method for the quantitative determination of hydrogen cyanide in cigarette smoke using pulsed amperometric detection

The analysis of hydrogen cyanide in cigarette smoke has been carried out for many years. The toxicity of hydrogen cyanide has long been recognised and it is included in the majority of lists of harmful smoke compounds. The most commonly used method for the measurement of hydrogen cyanide is continuous flow analysis with colorimetric detection. A new method is described using ion chromatography with an electrochemical detector in the pulsed amperometric mode of operation. This technique combines high selectivity and ease of operation compared with continuous flow analysis. Also it does not require the use of potentially toxic organic reagents. Details of the new method are discussed including the detector waveforms needed for cyanide analysis. Validation parameters such as precision, accuracy, repeatability and limits of detection will be presented.

Typical data will be given comparing yields of hydrogen cyanide measured using the ion chromatographic method and the colorimetric method for Kentucky reference cigarettes and the CORESTA monitor cigarette. Two methods of trapping hydrogen cyanide in whole smoke will also be discussed, impingers and simple granular traps.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 16

Higher yield product smoking, are there yield errors inherent when using a mechanical smoking machine?

Cigars when smoked using CORESTA Recommended Methods Nos. 64/65 commonly give greater yields than cigarettes smoked under the corresponding ISO regime. Analytical cigar smoking is characterised by larger puff counts and an overall longer smoking time than the cigarette equivalent. It has been posited that pulling air and vapour phase through the Cambridge filter pad as it becomes increasingly loaded can result in reduced trapping efficiencies of the pad and so reduce the apparent yield of the product under test.

An examination of "breakthrough" was made for different yield products using two Cambridge filter pads in series. The influence of pad area on trapping efficiency and retention was further examined using the same system and the reduction in vapour loss through use of a small diameter pad, relative to the larger pad, plotted against nominal yield.

The effect of puffing (clearing puffs) on a pre-loaded Cambridge filter pad was examined for different loadings, different puff volumes and different pad diameters.

A recommendation for modification of the method used when smoking cigars is presented with an estimate of the apparent impact this will have on measured TPM. The consequences and applicability of these changes for Canadian method intense smoking are explored.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 07

Relationship between puff intensity and smoke yields from a series of different smoking regimes

Imperial Tobacco Group data from two commercially available cigarettes, sold in the early 1980s and having 0 and 28% filter ventilation was presented this year at an ISO TC126 Working Group 10 meeting. The products had been smoked at different puffing frequencies and puff volumes. By using the parameter of puff intensity (expressed in mL/min) as the product of puff volume and frequency it was shown that NFDPM, nicotine and CO yields could be predicted for any smoking regime. Some of the WG10 members asked whether similar results would be obtained with currently available cigarettes. Data is presented on such products with both ventilated and non-ventilated commercial cigarettes from Morocco and Arabic countries. Cigarettes were smoked by 16 machine smoking regimes (Filtrona, SM450) covering 17.5 mL to 70 mL per puff, frequencies from 1 to 3 puffs per minute and a constant 2 second puff duration. Data is put into context with currently used or proposed smoking regimes and with human smoking data obtained on these two products.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 10

A method for the determination of NNAL, iso-NNAL & NNA in smokeless tobacco products

The presence of 28 compounds in smokeless tobacco products (STPs) was summarized in IARC Monograph 89, including nitroso compounds such as NNN (N-nitrosonornicotine), NNK (4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanone) and NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol). Although there have been a significant number of studies that analyzed the contents of NNN and NNK in STPs, NNAL, iso-NNAL, and particularly NNA (4-(N-nitrosomethylamino)-4-(3-pyridyl)-1-butanal) have received significantly less attention.

Historically NNAL and iso-NNAL have mainly been determined using non-specific techniques such as GC-TEA, combined with extensive sample clean-up and concentration procedures that may impact on the accuracy of data. No published method for NNA in STPs is currently available. Therefore a sensitive and specific method for these three analytes was developed and validated, in which samples of smokeless tobacco products were spiked with deuterated NNAL, iso-NNAL and NNA as internal standards. After the internal standards were allowed to soak into the samples, water was added and the samples were left to fully hydrate and swell. The samples were then extracted by subsequently adding methanol, shaking vigorously and centrifuging the extract.

Aliquots of the supernatants were analyzed directly for NNA by HPLC-MS/MS, because it was shown that NNA decomposes during the clean-up of the samples. Further aliquots were acidified with 2% formic acid in order to fully protonate the analytes, after which these aliquots were cleaned-up by cation exchange SPE. The analytes were deprotonated and eluted from the cartridges with 5% ammonia in methanol, after which these eluents were analyzed for NNAL and iso-NNAL by HPLC-MS/MS.

The method was demonstrated to be fit for purpose for the quantification of NNAL, iso-NNAL and NNA in various different STPs in the concentration range for matrix samples of 31.3-625 ng/g for NNAL and iso-NNAL and 1250-12500 ng/g for NNA.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 03

Statistical simulation to evaluate the effects of the number of laboratories and replicates on the precision of measurement method

In an inter-laboratory comparison, different laboratories measure particular characteristics of a product. For cigarettes, smoke analyte yields in one or more homogeneous samples are measured under ISO-defined conditions. The comparison of different laboratories that perform comparable analyses with their own individual method is often called proficiency testing or laboratory performance studies. It is an essential part of the requirements for laboratory accreditation. Inter-laboratory comparisons are also used by groups intending to produce an analytical method to be used in a broader environment, such as international standards for a commercial interface; methods approved by regulatory bodies; official methods produced by reference committees or other types of international organisations. In this case the terms collaborative study or collaborative trial are currently used.

The required final results are the precision of the method in terms of repeatability and reproducibility. A sufficient number of participating laboratories is required to provide robust values. However, different organisations recommend different numbers of participating laboratories. For example ISO standards (ISO 5725-1, 1994) recommend 8-15 laboratories; IUPAC specify an absolute minimum number of 5 and AOAC recommend a minimum of 8. Different organisations also have different approaches towards the replication of experiments, for example, ISO does not specify the number of replicates.

Additional smoke constituent testing and regulation is likely in the future and further awareness and understanding of the statistical interpretation of generated data is required to facilitate testing based on sound scientific foundations. In this paper, we described the effects of the number of laboratories and replicates on the estimate of precision. We also examined the effect of the ratio SDR/SDr on the estimated confidence intervals in order to investigate the contribution of number of replicates on the reproducibility. We demonstrated that for a high ratio the minor improvement of within-laboratory precision, obtained by increasing the number of replicates, is totally diluted in the between-laboratory precision.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. STPOST 10

Determination of oxides of nitrogen (NO_x) in analytical smoking Part 1: Complexities in calibration methods

Regulatory demands for reporting of larger numbers of analytes in tobacco and tobacco smoke has rekindled interest in quantification of oxides of nitrogen (NO_x) in tobacco smoke. Confounding measurement accuracy is the shortened lifetime of free NO within the tobacco smoke vapour phase matrix and the relationship between experiment equipment design and the calibration process.

Various arrangements have been devised where by standard smoking machines have been converted to capture and analyse NO_x using bag systems but this approach has inherent compromises between accuracy and simplicity. In contrast the design of commercially available analytical smoking engines in a puff by puff manner were unsuitable for truly accurate measurements of NO_x so an optimised smoking platform was devised and built using the chemiluminescent method for NO/NO_x analysis.

The accuracy of measurement of calibration gas introduction at three points, at analyser, through vapour path and through whole smoke path were compared for a clean system and one where smoking had been conducted. The influence of vapour phase condensate within the system on the calibration and by inference the analysis of smoke vapour was investigated and an estimate formed of the error introduced by calibration by these three different paths.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 54

Preparation of hemoglobin immobilized self-assembling nanoporous filter material and its application for selective reduction of harmful components in cigarette smoke

To reduce harmful components in cigarette smoke, 3 kinds of methoxy poly(ethylene glycol)-block-poly (D, L-lactic acid) (PEDLLA) nanoporous hydrogels (PNH) were prepared and characterized by ^{1}hydrogen-nuclear magnetic resonance spectrometer (^{1}H-NMR), infrared spectra (IR), transmission electron microscopy (TEM) and laser particle size analyzer. The results showed that the polymer had designed chemical structure with self-assembling character that appeared as hollow nanoporous particle with particle size around 100 nm. Hemoglobin was successfully immobilized into the hollow nanopore of PNH to provide hemoglobin immobilized nanoporous hydrogel (HINH). The total particle matter (TPM), tar, nicotine, CO and HCN in mainstream smoke of cigarette with a HINH filter were all decreased. According to different amounts of hemoglobin immobilized in hydrogel, CO decreased by 13.41% to 28.05% and HCN decreased by 13.27% to 21.24%, while cigarette smoking quality was only slightly affected. With significant selective reduction effects on harmful components in cigarette smoke, HINH has a good application prospect.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 01

Approaches for the development of test methods in a regulated environment - tortoises and hares, purism, procrastination and pragmatism

The development and application of standardised, fit for purpose test methods is a pre-requisite for the effective regulation of products. Without such test methods it is impossible to obtain the data needed to fully characterize product variability, to define appropriate product standards and to monitor compliance with the standards. The process by which test methods are developed and demonstrated to be fit for their intended purpose must be rigorous and should include the relevant stakeholders and end users of the methods.

Test methods are required to fulfil a range of purposes that includes product development, regulatory submission, quality control and monitoring of compliance. The level of analytical information required can vary from feasibility, through screening or semi-quantitative to confirmatory data. Technical and logistical constraints, including the required capability, laboratory capacity, scope of application, resource requirements and ultimately cost, must also be anticipated and addressed during method development. For reasons of cost effectiveness and data correlation, it would be advantageous to harmonise methods so that many analytes can be determined in a single sample. The definition of a framework of testing purposes, appropriate technical performance standards and fitness for purpose is a significant challenge.

Technical standards and guidance relating to the development of test methods and the assessment of their fitness for purpose are numerous, leaving scope for selective application or interpretation. The presentation will demonstrate and contrast possible procedures for the development of standardised test methods and suggest a pragmatic approach.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 11

Determination of *N*-nitrososarcosine in tobacco and tobacco products using Isotope Dilution Liquid Chromatography Tandem Mass Spectrometry

A simple, fast, selective, and robust analytical method for the determination of *N*-nitrososarcosine (NSAR) has been developed based on Supported Liquid-Liquid Extraction (SL-L) coupled with Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). To our knowledge, this is the first LC-MS/MS method for the analysis of NSAR in tobacco. In the past, NSAR has been determined mainly by gas chromatography with a thermal energy analyzer (GC-TEA), using a variety of sample cleanup and derivatization steps. The LC-MS/MS method described here, using an isotope labeled internal standard, adds greater confidence for the determination of NSAR in complex sample matrices such as tobacco. It also uses a relatively simple extraction (2% formic acid) and cleanup procedure compared to existing methods. The new method has been validated using three different control samples (Kentucky reference KY3R4F, smokeless research tobacco control products 1S2 dry snuff and 2S3 moist snuff), and the method exhibits good linearity ($R^2 \geq 0.999$) over a wide range of concentration (3-2400ng/g). The limit of detection (LOD) is 27.3 ng/g and the limit of quantification (LOQ) is 91.0 ng/g. While no NSAR was detected in KY3R4F, and only a low amount in 2S3, a relatively larger amount of NSAR (550.5±43.7 ng/g, n=100) was found in the dry snuff reference tobacco 1S2. The precision of the method is good (with a relative standard deviation of 7.94% for 1S2 samples), and the method is robust in a commercial laboratory environment, and can be applied to the determination of NSAR in cigarette tobacco and smokeless tobacco products.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 51

Selective removal of phenol from cigarette smoke by special CPF filter made from modified grooved cellulose plug paper

In this study special combined performance filters (SPCF) were prepared by adding polyethylene glycol/acetylpropionic acid/trithylene glycol diacetate to cellulose plug paper. The effect of SPCF on the selective removal of phenol from cigarette smoke was studied in detail. The results showed that SCPF filters modified by a single additive or multi-additives had a high ability to selectively remove phenol in tobacco smoke. The more the additives on cellulose paper, the higher effect on the percentage phenol removed. It was also found that SCPF filters gave a high and permanent level of phenol removal when they were aged for 8 weeks. Optimal results were obtained when the additive acetylpropionic acid on cellulose paper was 9±0.5 g/m^2, where phenol was significantly reduced up to 33.31%, whereas the delivery of tar was nearly unaffected (only reduced by 2.61%). In addition, *in vitro* toxicology tests of cigarette smoke indicated that: SCPF filter modified by acetylpropionic acid was helpful to improve the safety of smoke. Moreover, the sensory assessment of samples indicated that the samples with SCPF filters kept the original taste and style. This finding is significant for the selective removal of harmful components in tobacco smoke.

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CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, 2011, abstr. ST 13

Determination of tobacco specific N-nitrosamines in smokeless tobacco products by liquid chromatography-tandem mass spectrometry

Tobacco specific N-nitrosamines (TSNAs) are the most abundant carcinogens identified in smokeless tobacco products. The accurate determination of TSNAs is significant to assess the health risk of smokeless tobacco products. A liquid chromatography-electrospray ionization mass spectrometry (LC-ESI MS/MS) method for the simultaneous determination of four TSNAs in smokeless tobacco was developed, and the TSNA contents in 71 smokeless tobacco product samples of different brands and types were determined with the developed method. The samples were extracted with ammonium acetate and analyzed without further cleanup. The results showed that: 1) the detection limits of N-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N-nitrosoanatabine (NAT) and N-nitrosoanabasine (NAB) were 0.03, 0.08, 0.03 and 0.02 ng/mL, respectively; the recoveries ranged from 93.17% to 105.25% with the precisions of 2.03% to 4.80%; 2) the contents of total TSNAs ranged from 0.54 to 29.35 µg/g with the average of 4.68 µg/g; there was a significant difference between the contents of TSNAs in smokeless tobacco products of different brands; 3) the developed method featured higher sensitivity and specificity and was suitable for the quantitative analysis of TSNAs in smokeless tobacco products.

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The yields of aromatic amines in the pyrolysis of solvent-extracted tobacco

Tobacco smoke is a complex mixture of a wide variety of chemical compounds, including aromatic amines (ArAs) such as 2-aminonaphthalene (2-AN) and 4-aminobiphenyl (4-ABP). A previous study has estimated that proteins in tobacco highly contribute to the pyrolytic formation of 2-AN and 4-ABP, by evaluation of the increase of their pyrolytic yields with added bovine serum albumin as a model protein to tobacco. The aim of this study is to estimate the magnitude of each nitrogenous compound in tobacco, including endogenous proteins, for the pyrolytic formation of ArAs. Residues obtained by the sequential extraction of cut tobacco (CTRL) were prepared, and the changes of ArAs yield in pyrolysis by solvent-extraction were evaluated.

Burley type CTRL was sequentially extracted with CH_2Cl_2 followed by H_2O. The mixture of extract solution and residue was separated by filtration at each extraction step. Each sample including CTRL was non-isothermally pyrolyzed from room temperature to 800 °C (ramp rate; 1,000 °C/min.) under an inert atmosphere. ArAs collected on a glass filter during pyrolysis were analyzed by GC-MS after solid phase extraction and derivatization.

The results indicated that CH_2Cl_2 extraction did not change the yields of either of the ArAs significantly. The H_2O extraction decreased the yields of 2-AN and 4-ABP by 25% and 40% of the yields in CTRL pyrolysis, respectively. The final residue obtained by the H_2O extraction yielded 2-AN and 4-ABP over 50% of the yields from CTRL pyrolysis. Another type of tobacco, flue-cured, was also investigated with the same method as above, and similar results were obtained.

These results suggest that 2-AN and 4-ABP would form from water-soluble and insoluble nitrogenous compounds in tobacco under inert pyrolytic conditions. We will discuss the results obtained by pyrolysis under oxidative conditions.

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Application of thermal time model in studying changes of aroma components in cigarettes during storage

Thermal time is adopted to research the relationship of plant growth with climate, etc. A thermal time linear regression model for studying the changes of aroma components in cigarettes was developed based on the effects of different storage temperatures (from -5 to 50 °C). The results of ANOVA analysis and discriminant analysis showed that 20-30 °C was the initial temperature range wherein the total contents of volatile components in cigarette began to decrease. The methods were applied to analyze the changes of volatile organic compounds in cigarettes of three brands stored in two cities for two years. The results indicated that higher temperature could accelerate the decrease of aroma compounds and the quality of the same brand samples stored in different environments changed differently. Thermal time model is an effective method to discriminate the seasonal change rules of cigarette quality during storage.

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Analysis of vapour phase components in mainstream cigarette smoke by bag sample-thermal desorption-gas chromatography/mass spectrometry

A method for the analysis of vapour phase (VP) compounds in mainstream cigarette smoke (MCS) was described. Cigarettes were smoked by a rotary smoking machine under the standard ISO smoking regime and the VP passing through a Cambridge filter pad was collected in a Tedlar® bag. On completion of smoking, the contents in the bag were sampled onto Carbotrap 300 adsorption tube containing 3 beds of adsorbents (Carbopack C, Carbopack B and Carboxen III). The tube was subsequently analyzed by an automated thermal desorption (ATD) system coupled with a gas chromatographer-mass spectrometer (GC/MS) using a VOCOL column. The operating parameters of ATD and GC was optimized. The VP of MCS produced by reference cigarette 2R4F and 4 other cigarettes was determined. The results indicated that: 1) The VP compounds in MCS were basically completely trapped by Carbotrap 300; 2) 67 components were identified; 3) The response of GC/MS was linear with the concentrations of 13 analytes in different ranges; 4) The relative standard deviations (RSD) of 67 components were less than 10%; 5) Satisfactory recoveries (80%-120%) were obtained with independent check standards; 6) The results for University of Kentucky reference cigarette 2R4F well agreed with reported values; 7) The deliveries of 2-propenenitrile and acetonitrile in MCS of blend type cigarette were higher than that of flue-cured type cigarette; 8) The VP compounds in MCS could be selectively reduced by activated carbon.

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A rapid procedure for determination of nicotine and its metabolites in serum of smokers with different smoking levels by liquid chromatography-tandem mass spectrometry

A novel chromatography-tandem mass spectrometry (LC-MS/MS) procedure was developed and validated for the simultaneous determination of nicotine, cotinine, *trans*-3-hydroxycotinine, nicotine-N-ß-D-glucuronide, cotinine-N-ß-D-glucuronide, *trans*-3-hydroxycotinine-N-ß-D-glucuronide, nicotine-N-oxide, cotinine-N-oxide, nornicotine and norcotinine in human serum. The extraction and enrichment of all the target analytes and corresponding deuterated internal standards from human serum were accomplished by using acetonitrile as protein precipitator and centrifugation. The liquid supernatant was reconstituted into pure water after the organic phase was almost dried down, and achieved baseline separation within 4 minutes by LC-MS/MS with atmospheric pressure chemical ionization (APCI). The total extraction recovery ranged from 80% to 104% with the exception that the recovery of cotinine-N-oxide was 76%-78%. The limits of quantification for all analytes were less than 0.83 ng/mL. Seventy smokers were employed to smoke different amounts of cigarettes, and their plasma, urine and saliva were analyzed, respectively. The data showed that the cotinine in serum afforded a mean value of 163.63±71.61 ng/mL and the average levels of *trans*-3-hydroxycotinine in serum were 24.81±15.52 ng/mL. The results of classification analysis showed that the content of all the metabolites in serum could approximately predict the smoking levels of smokers. The correlation and contribution degree analysis of these metabolites indicated cotinine was a good biomarker suitable for the determination of smoking levels of smokers. Furthermore, the correlation coefficient of cotinine content in serum and saliva was 0.82, while that in serum and urine was 0.76. There was a significant correlation among the cotinine levels in three body fluids.

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Studies on removal of harmful components from cigarette smoke by functional plug wrapper containing tourmaline composite materials

A new functional plug wrapper containing tourmaline composite materials (TCM) was prepared, and its effects on the deliveries of tar, CO and other harmful components in cigarette smoke were investigated. The results showed that the deliveries of tar and other harmful components had been efficiently reduced by physico-chemical adsorption and/or electrostatic adsorption due to the special properties of pyroelectricity, piezoelectricity and spontaneous polarization of TCM. When the coating rate of TCM on plug wrapper was 14±0.5g/m^2, tar and CO deliveries were reduced by 13.73% and 16.19%, respectively; the other harmful components, such as NO_x, carbonyl compounds, phenolic compounds, HCN, TSNAs and B[a]P were reduced by 25.21%, 14.17%, 12.07%, 31.67%, 9.36% and 20.34%, respectively. Moreover, TCM was helpful in improving the smoking quality of the cigarette, and the functional plug wrapper containing TCM had a stable performance for the removal of harmful components from cigarette smoke. The influence of TCM coating rate on the removal of harmful components from cigarette smoke was studied. The results indicated that the reduction rates of tar, CO, NO_x, HCN and B[a]P were significantly increased when TCM coating rate was raised from 11±0.5 to 14±0.5 g/m^2, whereas they were almost unchanged when the coating rate was raised from 14±0.5 to 16±0.5 g/m^2.

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