Determination of "Hoffmann Analytes" in Cigarette Mainstream Smoke. The Coresta 2006 Joint Experiment

by

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SUMMARY

Regulatory authorities are currently discussing the measurement of and imposition of ceilings on certain smoke analytes, the so called "Hoffmann analytes". However, as a prerequisite, the measurement methods and the tolerances around the measurements first need to be established.
In 1999, the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) set up a Task Force "Special Analytes" to deal with analytical methodology for measuring "Hoffmann analytes" under International Standard (ISO) smoking and to work towards the standardisation of methods. This paper describes the output and conclusions from a 2005–2006 joint experiment made within the Task Force representing laboratories currently able to analyse these compounds. Data were obtained on most "Hoffmann analytes" from reference cigarettes (2R4F and 1R5F), collecting data according to the existing methods used by the nineteen participating laboratories, in order to describe the within and among laboratory variability and to see which methods could most benefit from more rigorous standardisation work.
In some cases, the applied statistical analysis found that methods could not well differentiate the 1R5F and 2R4F cigarettes of differing 'tar' yield. This was explained, in part, by the broad range of methods used by the participating laboratories but also indicated that there were significant inadequacies in the choice of some methods or weaknesses in their application.
Results indicate that "Hoffmann Analyte" data are generally more variable both within and among laboratories than nicotine free dry particulate matter (NFDPM); nicotine and carbon monoxide due to their lower smoke yields. Accordingly, tolerances around methods adopted for regulatory purposes will need to be proportionally higher.
Methods for benzo[*a*]pyrene (B[*a*]P) and tobacco-specific nitrosamines (TSNAs), already taken to CORESTA recommended methods or ISO standardised methods through the efforts of this Task Force, give some of the most reproducible results, showing the value of this process. However, these data strongly suggest that even these analytes have much higher among-laboratory variability than for NFDPM, nicotine and CO and, based on the only two available one point in time studies, may need tolerances in the range of 35–45% for B[*a*]P and 26–55% for TSNAs, if they are to be measured for regulatory purposes.
The collected data is useful to participating laboratories for internal method validation and laboratory accreditation, and data comparisons with others allow laboratories to identify strengths and weaknesses in their current methods.
However, much work still needs to be carried out to take most of the methods towards standardisation. Although some fundamental differences or areas of concern around the methodology are discussed herein, they are not comprehensive and there may be others that need to be addressed before methods can be considered ready to take to a Recommended Method and/or to an ISO Standard. These methodological issues are being addressed in further CORESTA work within this Task Force. Smoke analytes with the highest variability found in this study and those analytes that are currently of highest regulatory interest are being prioritised and after further joint experiments, the results are intended to be published. [Beitr. Tabakforsch. Int. 24 (2009) 161–202]

ZUSAMMENFASSUNG

In einigen Staaten werden von Seiten der Überwachungsbehörden seit einiger Zeit die Messung und damit ver-

bunden Höchstmengen für eine Gruppe von Substanzen, den so genannten Hoffmann-Analyten (HA), die im Hauptstromrauch von Zigaretten enthalten sind, diskutiert. Aus diesem Grund ist eine Überprüfung der zur Zeit genutzten Analysemethoden zur Bestimmung der HA wie auch die damit verbundenen Messtoleranzen dringend angeraten.

Seit 1999 befaßt sich die Arbeitsgruppe der CORESTA „Special Analytes" mit der Entwicklung von standardisierten Methoden zur Messung der HA unter den Abrauchbedingungen der Internationalen Organisation für Normung (ISO). Die vorliegende Arbeit beschreibt die Ergebnisse einer Studie, die von November 2005 bis Juni 2006 innerhalb dieser Arbeitsgruppe durchgeführt wurde und umfasst die Bestimmung von 34 der 40 HA an den zwei Referenzcigaretten Kentucky 2R4F und Kentucky 1R5F.

Daten von 19 Labors wurden ausgewertet, wobei jedes Labor seine eigene Methode zur Bestimmung der HA verwendete. Mit Hilfe der Studie sollte die Variabilität innerhalb und zwischen den Laboratorien untersucht werden und weiterhin die Frage, ob auf der Basis des jeweils angewandten Analyseverfahrens ein statistisch gesicherter Unterschied zwischen den Referenzzigaretten ermittelt werden kann.

Die Ergebnisse der Studie zeigten, dass für die gemessenen HA die Daten sowohl innerhalb der Labors als auch zwischen den Labors generell stärker streuen als Messdaten, die für nikotinfreies Trockenkondensat (NFDPM), Nikotin oder Kohlenmonoxid (CO) bestimmt werden. Sollten Höchstmengen für diese Analyten von Seiten der staatlichen Überwachung erwogen werden, sind entsprechende Toleranzen zu berücksichtigen.

Bei der Auswertung der Ergebnisse zeigte sich weiterhin, dass für die Methoden zur Bestimmung von Benz[a]pyren (B[a]P) und den tabakspezifischen Nitrosaminen (TSNA) die besten Reproduzierbarkeiten ermittelt werden konnten, was sicherlich auf die schon durch ISO vorgenommene Standardisierung dieser Methoden zurückzuführen ist.

Dennoch liegen auch für diese Methoden die Varianzen zwischen den Labors wesentlich höher als die Varianzen für NFDPM, Nikotin oder CO. Dieses Ergebnis wird durch verschiedene CORESTA Ringversuche bestätigt. Für B[a]P wurden dabei Toleranzbereiche von ± 45% bzw. ± 35% bei der Referenzzigarette 2R4F ermittelt, während für die vier TSNAs die Toleranzen zwischen ± 26% und 55% liegen. Die beteiligten Labors können die Daten aus der Studie sowohl zur Methodenvalidierung nutzen als auch zur Bewertung von Stärken oder Schwächen ihrer derzeit verwendeten Methoden heranziehen.

Um die verschiedenen Methoden für die Hoffmann-Analyten zu standardisieren, bedarf es sicherlich noch einiger Anstrengungen. In der Auswertung der Studie wurden u.a. die Aspekte der methodischen Unterschiede näher beleuchtet und diskutiert. Dennoch konnten nicht alle Gesichtspunkte der einzelnen individuellen Methoden in dieser Studie umfassend behandelt werden, so dass man davon ausgehen kann, dass der Weg zur Erstellung einer CORESTA „Recommended Method" oder einer ISO Methode noch weiterer Arbeit bedarf. Rauchanalyten mit der höchsten Variabilität und solche, die gegenwärtig von hohem regulativen Interesse sind, sollten in zukünftiger Arbeit vorrangig behandelt werden. [Beitr. Tabakforsch. Int. 23 (2009) 161–202]

RESUME

Les autorités de normalisation discutent actuellement de la mesure et de l'imposition des plafonds sur certaines analytes de la fumée, connues comme « la liste Hoffmann ». Cependant, ce qui constitue une condition préalable, les méthodes de mesure et les tolérances des mesures doivent être établies.

En 1999, le Centre de Coopération pour les Recherches Scientifiques Relatives au Tabac (CORESTA) a installé un groupe de travail pour examiner la méthodologie analytique pour la mesure des composants de tabac de la liste Hoffmann, selon le régime de fumage de l'ISO avec le but d'une normalisation des méthodes. Cet article décrit les résultats et les conclusions d'une étude effectuée dans les années 2005 à 2006 par le groupe de travail, représentant des laboratoires actuellement capable d'analyser ces composants.

Les dix-neuf laboratoires qui ont participé à l'étude ont mesuré les teneurs de 34 des 40 analytes de la « liste Hoffmann » dans les cigarettes de référence 2R4F et 1R5F, selon les méthodes généralement utilisées. Le but de l'étude était de savoir si les différentes méthodes d'analyses permettent de faire une différentiation statistique entre les cigarettes 1R5F et 2R4F.

Les résultats indiquent que les données obtenues des analytes de la « liste Hoffmann » sont généralement plus variables inter- et intra-laboratoire que le goudron, la nicotine et l'oxyde de carbone à cause de leur rendement plus bas de la fumée. En conséquence, si les rendements de ces composants dans la fumée devraient être plafonnés en vue d'une normalisation, les marges de tolérances devraient être plus variable.

L'examination des résultats de l'étude montre également, que les méthodes d'analyse pour le benzo[a]pyrene (B[a]P) et les nitrosamines spécifiques du tabac (TSNAs), pour lesquelles des méthodes normalisées ont déjà été élaborées, permettent d'obtenir des résultats les plus reproductibles.

Cependant, ces données indiquent fortement que la variabilité inter-laboratoire (r) soit beaucoup plus haute pour BaP et TSNAs que pour goudron, nicotine et CO. Basé sur les seules deux études ponctuelles disponibles, les indications sont que les valeurs de tolérance de BaP même pour la cigarette 2R4F à plus haut rendement devraient être au moins en moyenne le ±35% des 2003 données de collaboration de CORESTA et en moyenne le ± 45% de l'étude courante. De même, pour quatre TSNAs individuels, les tolérances devraient être au moins dans la gamme moyenne de ± 35 à 55% des 2005 données de collaboration de CORESTA et le ± moyen 26 à 42% de l'étude courante. Les données rassemblées sont utiles aux laboratoires participants pour la validation interne de la méthode et les comparaisons de buts et de données d'accréditation avec d'autres permettent à des laboratoires d'identifier les points de force et de faiblesse dans leurs méthodes courantes.

Beaucoup de travail doit encore être accomplit pour avancer la plupart des diverses méthodes vers la normalisation. Bien que quelques différences ou soucis fondamentaux à l'égard de la méthodologie soient discutés ci-dessus, elles ne sont pas complètes et il peut y en avoir

d'autres qui doivent être adressées avant que les méthodes puissent être considérées au niveau de méthode recommandée et/ou de norme de l'OIN. [Beitr. Tabakforsch. Int. 23 (2009) 161–202]

PARTICIPATING LABORATORIES

INTRODUCTION

Certain smoke constituents have been measured for many years in 'one point in time' studies and such data have been described, for example, in UK Governmental sponsored reports in the 1980s and 1990s (1–4). More recently, Health Canada have mandated the measurement of 44 smoke emissions, the so-called "Hoffmann analytes", on an annual basis, for brands sold in Canada using methods posted on their website (5). The term "Hoffmann analytes" has been adopted by many industry and regulatory scientists as an acknowledgement of the work carried out by Dietrich Hoffmann and co-workers at the American Health Foundation. He identified, catalogued and published lists of biologically active substances in cigarette smoke, for example, as given in the reference (6).

Various 'one point in time' benchmark studies have also been requested by regulatory authorities (7–10) to compare commercial products in a specific market. There are also a limited number of small-scale within- and among-laboratory studies on "Hoffmann analytes" that can also be found in the literature describing both short-term and long-term variability (11–15) within reference or commercial cigarettes.

Recently, TobReg (16), a part of the World Health Organisation, have been discussing the regular measurement of certain analytes on a global basis and proposals for the imposition of ceilings on certain analytes on a per milligram 'tar' or per milligram smoke nicotine basis. However, the measurement methods and the tolerances around the measurements first need to be established within a rigorous standardisation process before this is practically feasible.

In 1999, CORESTA responded to the greater interest in "Hoffmann analytes" in the regulatory environment by working to standardise the methods for the measurement of benzo[$a$pyrene (B[a]P) and tobacco-specific nitrosamines (TSNAs). These analytes were chosen as a priority because of assumed regulatory interest and also because it was felt that their analytical methods would be reasonably straightforward to take to standardisation. However, this work took a considerably longer time than expected in order to achieve reasonable agreement in yields across all the participating laboratories when applying the same methodology. It was also taken into consideration, when the standard methodology was chosen, that the instrumentation should be available to most competent laboratories without incurring prohibitive costs. The recommended CORESTA methods are now available for B[a]P and TSNAs (17–18) and B[a]P has gone through the ISO standardisation process (19).

Although it is clear that the learning that has been achieved during these standardisation processes can be applied to other analytes, CORESTA felt that in the light of the probable length of time taken to bring further methods forward to Recommended Methods and/or to ISO standardisation it would be more useful to approach the issue in the manner described below as an initial step before embarking on further method standardisation.

It was agreed by CORESTA in 2005 that a broad survey of methodology used by participating laboratories should be undertaken and that data should be compared on a range of "Hoffmann analyte" smoke constituents obtained from

reference cigarettes according to existing methods used by participants. The objective of this joint experiment was to describe the within- and among-laboratory variability across a large number of "Hoffmann analytes" in order to see which methods already gave comparable data and were thus more suitable to proceed towards standardisation and which methods would most benefit from rigorous investigative work before embarking on standardisation. This work was carried out in laboratories with current expertise in these analytes.

Although it was recognised that a more intense regime may be introduced into the regulatory arena in the future (16), it was decided that the current ISO smoking regime (20) should be used in this joint experiment.

This paper describes the output from this joint experiment. Although some fundamental differences or areas of concern around the methodology are discussed herein, they are not comprehensive and there will be others that need to be addressed before methods can be considered ready to take to a Recommended Method and/or to an ISO Standard. These issues are being addressed in further work of the Task force.

EXPERIMENTAL

Overview of the protocol

The following work was organized within the CORESTA Special Analytes Task Force in the 2006 Joint Experiment. Proficiency testing using, for example, the Food Analysis Performance Assessment Scheme (FAPAS) allows participating laboratories to use their own methodologies for testing a reference material. This approach seemed inappropriate for cigarette smoke methods where the reference material was smoke and for smoke analytes that were generated *in situ*. Therefore, it was decided to obtain guidance from statistical experts on an appropriate experimental design and protocol for this joint experiment. The term 'Hoffmann analytes" has been used to describe the list of smoke components that Health Canada has mandated for annual testing on Canadian cigarette brands. The chosen "Hoffmann analytes" for this study were ammonia, four aromatic amines, B[*a*]P, eight carbonyls, hydrogen cyanide (HCN), nitric oxide (NO), four tobacco-specific nitrosamines (TSNAs), three semi-volatiles, seven phenols and five selected volatiles[a]. These are described in more detail in Appendix 1.

Toxic metals were omitted from the study because few laboratories were set up to carry out these analyses and even those that could undertake the analyses often found levels below the limits of quantification for many of the metals. The participants also decided, rightly or wrongly in hindsight, that it was inappropriate to include NFDPM, nicotine and CO in this study as these are covered within other CORESTA working groups.

A draft protocol was agreed with the objective to describe the variability of "Hoffmann analytes" as analysed over a range of laboratories. Each laboratory was requested to use its own in-house methods and agreed to share their methodologies. There was no rule given that the laboratories either had to have a certain level of accreditation or any other auditing of their methodology and suitability to partake in the study.

The 2R4F and 1R5F Kentucky reference cigarettes were the products chosen for testing and were sourced from a single batch isolated by the University of Kentucky to try to minimise product variability. Five replicates for each analyte in three independent experiments were required, providing a total of 15 results for each analyte. Participants were asked to follow the given smoking plans for rotary and linear machines.

It was requested that the five replicates should be run over one or two consecutive days and the three experiments should be run with a minimum of one week or longer in between each experiment. A full description of the protocol is given in Appendix 1.

An excellent response was given to this work by participants and results were obtained from 19 laboratories. The numbers of data sets received from laboratories, for individual analytes, ranged from 12 to 18 for the 2R4F cigarette and from 9 to 16 for the 1R5F cigarette. Some laboratories found levels below their limits of quantification when analysing 1R5F and so the number of data sets available for further analysis on the 1R5F cigarette is lower than for the 2R4F cigarette.

Overview of data

Some weight data was provided by 16 laboratories. The 1R5F cigarette had a mean weight of 0.845 g and the 2R4F cigarette had a mean weight of 1.058 g after standard ISO conditioning (21). For each cigarette type, the coefficient of variation (CoV) in measured mean weights across these laboratories was only 2% indicating that for this parameter at least, the product was fairly uniform.

Over 18,000 data points were obtained across all analytes. Mean values for each analyte were obtained across all laboratories and are shown in Appendices 2 and 3 for the 2R4F and 1R5F cigarette types respectively.

From analysis of these results, Appendices 4 and 5 show mean data for each analyte across all laboratories for each cigarette type along with standard deviations (SDs), CoVs, maximum (Max) and minimum (Min) mean values from participating laboratories, the ratio of maximum to minimum (max/min) values and the number of data sets for both 2R4F and 1R5F. This information gives an overview of all collected data prior to any data removal.

It can be observed from CoV values in Appendix 4 that for 2R4F cigarettes, the least variable data was observed for catechol and *N*-nitroso-nornicotine (NNN) whereas the most variable data was observed for quinoline and resorcinol possibly because these were being measured near to their limits of quantification (LOQ). The most extreme differences between the laboratory means were seen for acrolein, toluene, pyridine and styrene (from max/min data). This might be caused by methodological problems, poor calibration, or data transcription problems

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[a] Some of the "Hoffmann analytes" on the Canadian smoke emissions list have never or have not been consistently listed as important biologically active smoke constituents by DIETRICH HOFFMANN (6). For example, the following compounds are not included in the given reference e.g., 1-aminonaphthalene, 3-aminobiphenyl, propionaldehyde, butyraldehyde, MEK, acetone hydroquinone, resorcinol and the three cresols. It should be noted that *m*-cresol and *p*-cresol are measured together and given as a combined yield in this and many other studies.

at the laboratory before results were sent for statistical analysis. In some cases, a high or low value resulted from just one laboratory performing markedly differently than the rest, creating a false impression of higher variability. For 1R5F, the least variable data was seen for catechol and acetaldehyde and the most variable data seen for pyridine and formaldehyde as depicted in Appendix 5. The most extreme differences (max/min) were found for 1-naphthylamine, toluene, pyridine and styrene, again this might have been due to methodological or data transcription problems.

Statistical analysis

The statistical functions 'r' and 'R' are properly applied within collaborative studies using the same methodology across all laboratories to provide estimates of the repeatability within-laboratory and the reproducibility among-laboratories respectively. However, data in this joint experiment was obtained at one point in time by laboratories each using their own chosen method.

Data analysis in this report cannot provide estimates of reproducibility and repeatability. However, an analysis was carried out to describe statistically the within-laboratory variability of analytes, to identify analytes that give similar average yields irrespective of the laboratory method used, and to describe those analytes which show a higher variability among laboratories thereby indicating those methods that are less specific or in greater need of standardisation.

An attempt was also made to identify any inherent parameters in the methodology that might lead to marked yield differences among laboratories (i.e. effects of smoking machines and number of cigarettes per replicate). Evaluations were made by the various statisticians, based on ISO Standard 5725 (22), and the differences in their approaches are set out in Appendix 7.

Data removal: It was agreed that it would be difficult to make a judgement on whether any particular data were really outliers and so it was decided that all data should be retained wherever possible. However, certain data were removed on the following basis.

a) Analytes where fewer than 10 results of the possible 15 were obtained from a laboratory. This ensured that each laboratory's mean was based on data from at least two experiments to give reasonable precision.

b) Some data had certain irregularities or did not strictly follow protocol. For example, laboratory 11 was excluded because it did not separate out its data into the three different experiments required in the protocol.

c) Some 1R5F datasets were reported with all values less than the LOQ and so the 2R4F values had to be excluded for these datasets when doing certain comparisons between 2R4F and 1R5F (see below).

Details of the data removed prior to statistical analysis are shown in Appendix 6.

Within-laboratory variability: The statistical analysis estimated within-laboratory variability values (r) among replicates and experiments found within the various participating laboratories. Although it used the same statistical equations, this approach could not properly estimate repeatability within-laboratory because each laboratory did not use a standardised methodology. The equations defining r and its CoV, and r values obtained for both the 1R5F and 2R4F for each of the studied analytes are given in Appendix 7 and are based on statistical equations described in ISO 5725 (22).

From Appendix 8 it was observed that CoV (r) values were generally lower for 2R4F than for 1R5F across all analytes and that CoV (r) was generally higher for "Hoffmann analytes" than for nicotine free dry particulate matter (NFDPM). There was no statistical evidence in this study that compounds measured in μg/cig were any less variable within-laboratories than those in ng/cig.

Typical data on NFDPM variability are included in Appendix 8 for comparative purposes and are taken from another recent CORESTA study (23). As expected, the value for within-laboratory variability for NFDPM was lower than for most of the 35 "Hoffmann analytes" under study.

Among-laboratory variability: Similarly, the statistical analysis used the same method to estimate among-laboratory variability values (R) as used to more properly estimate reproducibility among-laboratory in collaborative studies using the same methodology across laboratories. Although it used the same statistical equations, this approach could not properly estimate reproducibility among-laboratory because each laboratory did not use a standardised methodology. The equations defining R and its CoV and R values obtained for both the 1R5F and 2R4F for each of the studied analytes are given in Appendix 7 and are based on ISO 5725 (22).

From Appendix 9, it was observed that among-laboratory variability is higher for 1R5F than 2R4F but these differences were not as distinct as those in Appendix 8 depicting within-laboratory variability. It was also observed that CoV (R) values were much higher for the 35 "Hoffmann analytes" under study than for NFDPM derived from the previous CORESTA study (23) using the ISO standardised smoking method (20).

Well established tolerance values within 95% confidence limits are given in ISO 8243 (24) for NFDPM, nicotine and carbon monoxide when measuring over a period of time and at one point-in-time. Differences in yield measurements up to the tolerance values can be expected from any two laboratories on similar samples. These tolerances are $\pm15\%$ (period of time) and $\pm20\%$ (point of time) for NFDPM and nicotine and $\pm20\%$ (period of time) and $\pm25\%$ (point of time) for carbon monoxide. For lower yielding products these tolerance figures are transformed to absolute numbers for minimum tolerances, that is ±1.0 mg for NFDPM, ±0.1 mg for nicotine and ±1.5 mg for carbon monoxide.

It was therefore not unexpected that some very high values for %CoV were noted for the low yielding analytes (Appendix 10) particularly for the 1R5F cigarette when comparing data among-laboratory. This is in contrast to findings for the within-laboratory variability.

Differentiation between 2R4F and 1R5F reference cigarettes: It is clear that all the "Hoffmann analytes" will need to be standardised if they are to be used for regulatory

purposes. However, as a first step, one of the main objectives of this study was to identify which analytes give similar results irrespective of the laboratory method used, and those analytes which show a higher variability among laboratories thereby highlighting the greater need for a more detailed evaluation before they progress to full standardisation.

The assessment of both the method stability and the expertise applied in the different laboratories used in this study is based on the similarity / dissimilarity of the data among laboratories. The statistical analysis of the effectiveness with which the array of laboratory methods used in the study for each analyte was able to differentiate between the 1R5F and 2R4F cigarettes is the means that was used to reach this objective. The statistical assessment is made on the basis that each of the two reference cigarettes would be tested by two different laboratories, together with the assumptions that each analyte is roughly correlated with NFDPM for these two reference cigarettes. Here, it is noted that on the NFDPM scale these two samples are distinctly different, with average yields of approximately 1.7 and 9 mg/cigarette, respectively.

Figures 1–11 show the ability to discriminate between the two types of cigarette (1R5F and 2R4F). This is given as an estimate of the within laboratory variability (r) on the left side of the graphs and as an estimate of the among laboratory variability (R) on the right side of the graphs. If data for the r intervals is shown to overlap then it is not possible to discriminate even within at least one of the laboratories between these two cigarettes. However, most of the laboratories could differentiate between the two cigarette types in this way as also shown in Appendix 11.

1R5F yields of certain analytes may be measured at high levels in one laboratory and these yields may be in the same yield range as relatively low 2R4F yields measured in another laboratory. This leads to overlapping R values from which it can be concluded that the methods applied in at least two laboratories cannot differentiate between 1R5F and 2R4F. A summary of the statistical approach used is given in more detail in Appendix 11. A summary of the extent to which each analyte was able to distinguish between 1R5F and 2R4F cigarettes is illustrated graphically in Appendix 12. The vertical axis defines the smallest difference between the mean yields for 2R4F and 1R5F (samples A and B) that can be differentiated, with 95% confidence, when each sample has been tested by separate laboratories. Axes have been normalised to % CoV of R (Mean 2R4F – 1R5F) to allow plots of all analytes on one graph.

Points on the y-axis which are ≤ 100 indicate that for these analytes it would be possible to differentiate, with 95% confidence, between 1R5F and 2R4F cigarettes among laboratories using their current methods. However, there is still a variability around the data and there is still a need for rigorous method standardisation.

For analytes where the points on the y-axis are ≥ 100, it would not be possible to differentiate between 1R5F and 2R4F cigarettes using their current methods. For 9 of the 34 analytes there would appear to be a greater need to investigate in the most detail some of the factors involved in this variability before moving on to standardise a method and also to obtain proper repeatability and reproducibility data in properly conducted collaborative studies.

▸ B[*a*]P and the TSNAs, which have recently been subjected to laboratory method standardisation, gave some of the best yield differentiation. All individual phenolics, also gave good differentiation, except resorcinol.
▸ Carbonyls, HCN and NO yields differentiated well or moderately.
▸ Analyte groups for which most individual analyte yields were poorly differentiated were ammonia, semi-volatiles, aromatic amines and selected volatiles.

Possible effects on variability of various methodological features: a) Number of cigarettes per replicate – For most methods, at least 5 cigarettes were smoked per replicate. However, there are some in-house established methods where a lesser number were smoked such as for the carbonyl group. There was a statistically significant relationship between the standard deviation (SD) and the number of cigarettes smoked per replicate as seen in the graphical plot for acetone in Appendix 13.

Increasing the minimum number of cigarettes per replicate for each analyte may minimise the variability between replicates for some analytes. There would seem to be a case for recommending a minimum number of cigarettes smoked per replicate for each analyte in order to help to minimise the variability between replicates.

b) Smoking machines and other methodological features – The possible effects on yields of different smoking machine types and other methodological features were investigated. Due to the number of interacting factors, the effects, if any, of smoking machine type could not be detected. Total particulate matter (TPM) yields relating to specific analytes were not supplied by the participants in every case or could not be supplied due to methodology restrictions and so were not analysed statistically. TPM generation may be affected by different methods and may be a contributing factor to variability. Puff count is often a good indicator of good smoking practice and the correct conditioning of cigarettes. The mean TPM and puff count data, provided by participating laboratories, are summarised in Appendices 14 and 15. It was observed that some of the highest or lowest analyte yields within datasets were associated with some of the highest or lowest TPM yields respectively.

Other than the specific observations discussed under the following section, it was noted that within the current study it was not possible to identify any general features having a statistically significant effect on yields. It was not the intention of this work to evaluate methodologies in detail although some observations have nonetheless been described in the following section. More detailed investigation of specific aspects of methodologies will need to be carried out and reported in further Task Force work.

INDIVIDUAL METHODOLOGY DISCUSSION
AND STATISTICAL EVALUATION

Overview of statistical handling of data

A brief overview description of the methodology employed at each laboratory is provided below and the related Appendices for each of the groups of analytes. As the basis
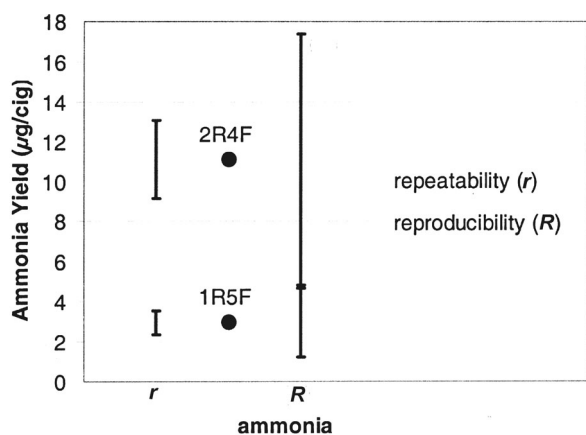
Figure 1. Summary of ammonia yields and their variability

for continual method improvement at each laboratory and to move towards better standardisation of methodology, detailed information was shared between participants and is discussed briefly.

As mentioned earlier, Appendix 12 indicates the relative status of all the analytes with respect to the capability of current methods differentiating between the two reference cigarettes. In the following subsections more details are given for each analyte group including pictorial representations of the estimated variability (r and R values). Where the lines representing r and/or R for the two reference cigarettes overlap, all participating laboratories were requested to investigate whether their yields were among the most extreme and, if so, to consider future modifications to their methodologies.

The estimated statistical r and R values in this section were obtained in a slightly modified way to those shown in the Experimental section but any differences still lead to the same conclusions by both methods. This was simply because of the slightly different statistical approach used by two different statisticians. The equations are also given in Appendix 7 and are based on ISO 5725 (22). Analyte groupings are considered simply in alphabetical order.

Ammonia methodology

Twelve laboratories provided ammonia data and their choice of methodology is shown in Appendix 16. A summary of mean yields and range of maximum and minimum yields for each laboratory is shown graphically in Appendix 17.

Participants raised questions on the effect on yields of certain methodology features such as linear versus quadratic calibration; the time and temperature dependency between smoke collection and analysis: because data indicate that ammonia levels may increase with time, and the influence of the volume of the impinger solution.

Eleven laboratories applied ion chromatography (IC) and one laboratory (Number 3) used photometric methodology. The latter method could possibly overestimate the yield because the method is not specific. This hypothesis is supported by the observed higher yields from this laboratory. The mean yield for 2R4F was 11.5 µg/cig, (excluding Laboratory 3 = 10.2 µg/cig) and the mean for 1R5F was 3.1 µg/cig (excluding Laboratory 3 = 2.8 µg/cig).

Figure 1 shows the mean yields and r and R values for each cigarette type. Because R overlapped, it was concluded that it may prove difficult to always detect differences between samples among participating laboratories, if both of the different methodologies continue to be employed. It should be noted that no methods were excluded in any initial evaluation and the reason for the overlapping in this case may be that the photometric determination is less specific and less suitable methodology.

Aromatic amines methodology

Appendix 18 summarises the methodology used in the 13 contributing laboratories. The trapping systems incorporated the use of either Cambridge filter pads (CFPs) for 11 of the 13 laboratories or impingers for Laboratories 3 and 12. The amines were mainly reacted to form pentafluoropropionic acid (PFPA) derivatives although Laboratories 5 and 6 formed the heptaflurobutyric acid (HFBA) derivatives. The clean-up was mainly based on liquid-liquid partition, followed by derivatisation and solid-phase extraction on florisil. The detection and quantification was carried out by gas chromatography-mass spectrometry (GC-MS) with single ion monitoring (SIM).

The mean, maximum and minimum yields for each of the four individual amines are given in Appendices 19 and 20. Their relative yield patterns were very similar across most laboratories. However, a different pattern was observed for the 4 individual amines from Laboratories 3, 5 and 18 for the 1R5F cigarettes.

It was noted from the information provided by participants that some laboratories are running at LOQs that are quite near to the measured smoke yields, especially from the 1R5F cigarette.

There was variation of 2R4F yields among laboratories, but no indication that differences in results were caused by the variations in methods described previously. Laboratory 3 had significantly lower 1R5F yields for all amines, and although not supported by Laboratory 12 data, it raised the question of the relative effect of impinger vs. CFP traps.

In Figure 2, the R values were overlapping or were close to overlapping for each of the four aromatic amines. Thus, it may prove difficult to always detect differences between samples among the participating laboratories. In the case of 4-aminobiphenyl, some individual laboratories could not even discriminate between 1R5F and 2R4F as shown by the overlapping r values.

Benzo[a]*pyrene methodology*

Appendix 21 provides information on the methodology from the 17 laboratories that provided data for this analyte. Twelve laboratories used the CORESTA Recommended / ISO Method (17, 19), with GC-MS as the analytical tool. A further five laboratories used high performance liquid chromatography (HPLC) with fluorescence detection similar to the methodology initially investigated by the Special Snalytes Task Force.

Mean yield data and the range of minimum and maximum yields across laboratories are shown in Appendix 22 alongside the main methodological differences observed. Variability of results for both reference cigarettes was in
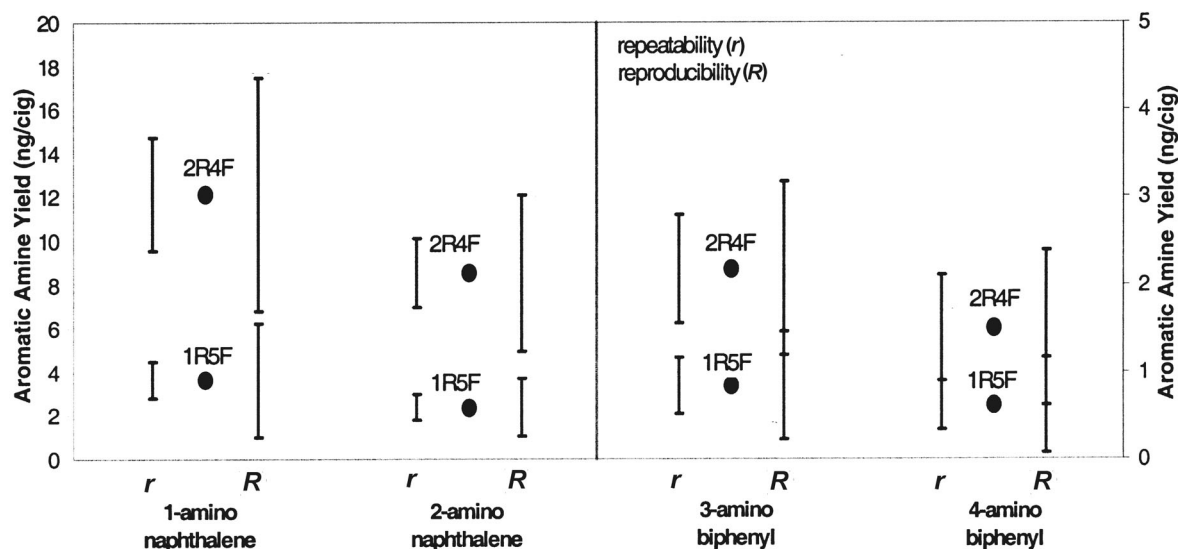
Figure 2. Summary of aromatic amine yields and their variability
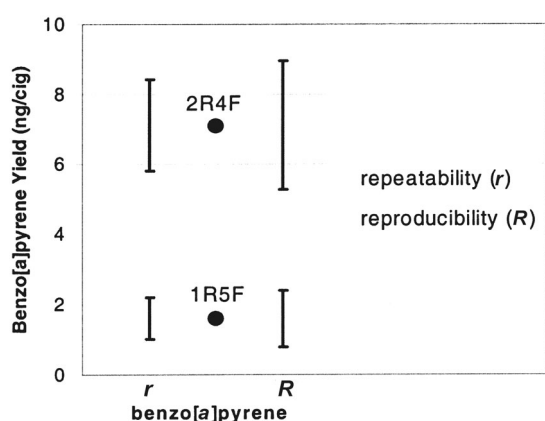
Figure 3. Summary of B[a]P yields and their variability

line with results previously obtained from joint Special Analytes Task Force experiments. The CORESTA method (17) with mean yield taken from the 2003 collaborative study for Kentucky 2R4F was 7.28 ng/cig with $r = 1.27$ ng and $R = 2.52$ ng. This compared extremely well with the mean value of 7.1 ng/cig with $R = 3.2$ ng obtained in the current study.

In Figure 3, good separation was found between the R values for the cigarettes of different 'tar' yields and R and r values were not too dissimilar.

This result clearly demonstrates the value of standardisation and the level of variation using standardised methods that might be expected from other smoke analytes if they were taken through a similar rigorous standardisation process.

Carbonyls methodology

A summary of the different methodologies used across laboratories is given in Appendix 23.

Twelve laboratories trapped smoke using only impingers. Two laboratories (Numbers 2 and 7) additionally trapped on CFPs. Two others (Numbers 18 and 19) trapped smoke on 2-4-dinitrophenylhydrazine (DNPH)-impregnated CFPs

and a further laboratory (Number 14) trapped smoke on activated silica after the CFP.

Fifteen out of the 17 laboratories carried out the derivatisation of carbonyls with 2,4–dinitrophenylhydrazine. One laboratory (Number 6) derivatised with 2-diphenyl-acetyl-1,3-indandione-1-hydrazone (DPAIH). Fifteen laboratories measured with high performance liquid chromatography-ultraviolet (HPLC-UV) or HPLC-DAD (diode array detection) and Laboratory 10 with liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS). Laboratory 14 applied colorimetric measurement using an auto analyser for formaldehyde and did not measure the other carbonyls for this collaborative study.

Mean, maximum and minimum yields and ratio patterns of the major carbonyls (acetaldehyde and acetone) are given in Appendices 24 and 25 and for the minor carbonyls (formaldehyde, acrolein, methyl ethyl ketone (MEK), propionaldehyde, butyraldehyde and crotonaldehyde) are given in Appendices 26 and 27. Major carbonyls compared well for both aspects. Laboratory numbers 11, 12 and 18 showed different yield patterns of minor carbonyls for the 1R5F cigarette. The highest yields for MEK were observed for Laboratory 12.

Although Laboratory 6 measured the yields of most carbonyls in line with most other laboratories, formaldehyde yields were lower with the DPAIH derivative used. An understanding of which hydrazone derivative is measuring formaldehyde correctly would seem particularly needed during method standardisation because the printing of formaldehyde yields is already mandated (25) on packets of Canadian cigarettes using Health Canada methods (5).

It can be noted that investigations (26) have suggested that the results for formaldehyde with DPAIH tended to be lower than some literature values for the Kentucky reference cigarettes obtained from laboratories using a DNPH solution made with unrecrystallised DNPH. Water is known to react with HMTA (hexamethylenetetramine) formed from the reaction of ammonia and formaldehyde in the vapor phase of smoke to form formaldehyde and ammonia. Thus, this referenced work found that to obtain accurate formaldehyde results every attempt must be made
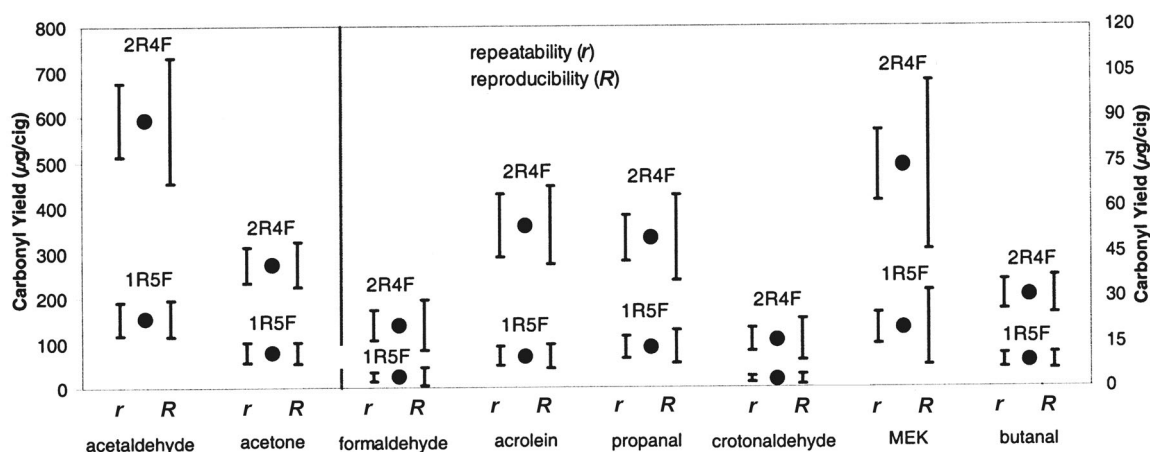
Figure 4. Summary of carbonyl yields and their variability

to reduce the water content of the trapping solution. The water content of unrecrystallised DNPH is approximately 30% by weight and, if used, will yield much higher formaldehyde results for the same cigarette when compared with recrystallised DNPH. Results obtained with recrystallised DNPH and DPAIH, the latter with no water added and with molecular sieves, are virtually identical for the same cigarettes.

It was also suggested that smoking more cigarettes will give a longer period before stopping derivatisation and has led to higher yields and a need for investigation and control of reaction time during method standardisation. Some participants noted that acetone yields may also be affected by the presence of water.

Some participants noted that crotonaldehyde is not well separated from other analytes in the chromatographic separation. It was discussed that resolution by C_{18} columns may depend on column suppliers and manufacturing lots, and has been found to be one of the factors affecting differences among laboratories.

In Figure 4, good separation was found between R values for the cigarettes of different 'tar' yields for all carbonyls. For formaldehyde, crotonaldehyde and MEK some improvements are still needed to enhance their method performance.

Hydrogen cyanide methodology

A summary of the different methodologies used across laboratories is given in Appendix 28. The methods of determination for this analyte were the most diverse of the ones considered.

A range of systems were used by the various laboratories to trap the smoke and the following observations were made.

▸ Eight laboratories used a CFP in combination with either 1 or 2 impingers.
▸ One laboratory collected smoke on the CFP and combined it with the contents of an Ascarite trap, containing sodium hydroxide-coated silica.
▸ One laboratory collected smoke not only on the CFP but also in a trapping unit with activated silica gel.
▸ Three laboratories simply collected smoke in two consecutive impingers.

▸ One laboratory collected smoke in a glass syringe after the CFP with individual puffs being sampled.
▸ One laboratory collected smoke in a glass tube containing sodium hydroxide solution.

Where it was used, the derivatisation step mainly involved HCN and chloramine T reacting with pyridine-pyrazolone or pyridine-barbituric acid to form a polymethine dye although in one case HCN was reacted with naphthalene-2,3-dicarboxaldehyde (NDA) and taurine.

Detection and quantification was undertaken by several different methods, such as colour reaction products with photometric detection in continuous flow analysis (CFA) in the majority of cases but also was quantified by

▸ Liquid chromatography - mass spectrometry (LC-MS) of the derivative or
▸ Headspace gas chromatography or
▸ Gas chromatography - flame ionisation detection (GC-FID) or
▸ Ion chromatography (IC) or
▸ Ion-selective electrode (ISE) determination.

Mean, maximum and minimum yield data obtained across the laboratories is shown graphically in Appendix 29 alongside major methodological differences. They show that there are no clear method-specific differences, although a relatively higher yield was obtained from both LC-MS/MS with NDA and taurine (Laboratory 10) and from trapping on activated silica gel (Laboratory 14). There is some indication that TPM yields might contribute to some extent to the relatively lower 1R5F yields for Laboratory 13 and higher 1R5F yields for Laboratory 14.

It can be noted that the printing of hydrogen cyanide yields has been already mandated on Canadian cigarette packets (25) by Health Canada methods (5) and a rigorously standardised method is required.

From Figure 5, it can be seen that it is possible to discriminate between cigarette types in the participating laboratories according to the statistical observations on r and R variability seen below.

Nitric oxide methodology

A summary of the different methodologies used across laboratories is given in Appendix 30. Mean, maximum and minimum yield results are summarised graphically in
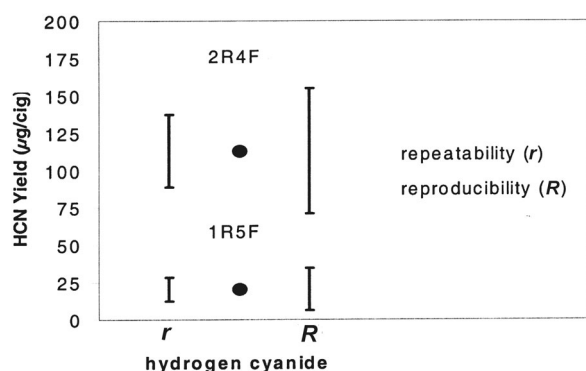
Figure 5. Summary of hydrogen cyanide yields and their variability

Figure 6. Summary of nitric oxide yields and their variability

Appendix 31. Some factors were identified as having the potential to cause among-laboratory variability such as the effect of the

- Analysis of 'fresh' smoke on a puff by puff basis compared to analysis of 'aged' smoke from all puffs mixed together at the end of the smoking run.
- Analysis of only whole puffs or the inclusion of partial (last) puffs.
- Analysis of NO versus total nitrogen oxides (NO_X).
- Smoke dilution within the collection chamber.

There is some indication that the relatively high 2R4F yield for Laboratory 4 is associated with a relatively high TPM yield.

From Figure 6 it can be seen that although R values were rather close to overlapping, it was possible to discriminate between cigarette types among the participating laboratories.

Phenolics methodology

A summary of the different methodologies used across laboratories is given in Appendix 32.

Two different methods were applied; one involved direct measurement of phenolics by HPLC-Fluorescence from CFP extracts and the other method involved BSTFA (*N,N*-bis(trimethylsilyl)trifluoroacetamide) derivatisation of the pad extract and GC-MS determination of derivatives.

Mean, maximum and minimum yield results are summarised graphically in Appendix 33. Yields and ratio of

patterns for all phenolics are given in Appendix 34. There is some indication that the relatively high 1R5F yield for laboratory 10 is associated with a relatively high TPM yield.

Yields and patterns of minor phenolics are shown in Appendices 35 and 36 and demonstrate that minor phenolics obtained from 2R4F by Laboratories 2 and 19 were different even though they used similar methodology. There was an inconsistent pattern of phenolics observed across laboratories from 1R5F for minor phenolics but no indication of method influence.

In Figure 7, R values overlapped for resorcinol. For other phenolics, it was possible to discriminate between cigarette types among the participating laboratories.

Selected volatiles methodology

A summary of the different methodologies used across 16 laboratories is given in Appendix 37. Laboratory 6 provided data using two different trapping systems. It was observed that the methodology was very similar.

Fourteen laboratories trapped smoke by impingers and CFPs. Of these, six laboratories smoked into one impinger trap; another fove into two consecutive traps and the other three had a set-up consisting of three or even four consecutive traps. The total volume of impingers ranged from 20 mL to 50 mL, with most applying 20 mL.

A further three laboratories (3, 5 and 6B) trapped vapour phase smoke in a Tedlar bag or a glass syringe. The participants highlighted the need to deactivate sites in the collection bag in this method.
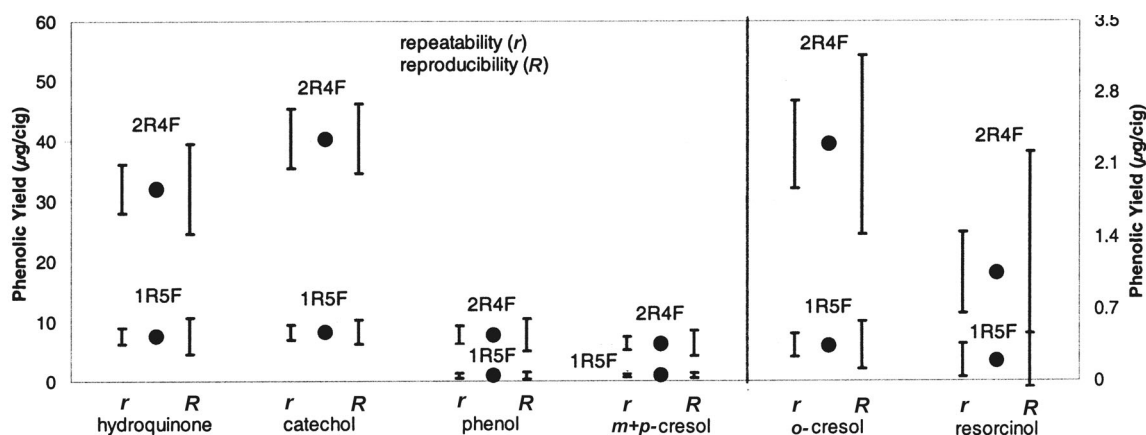
Figure 7. Summary of phenolic yields and their variability
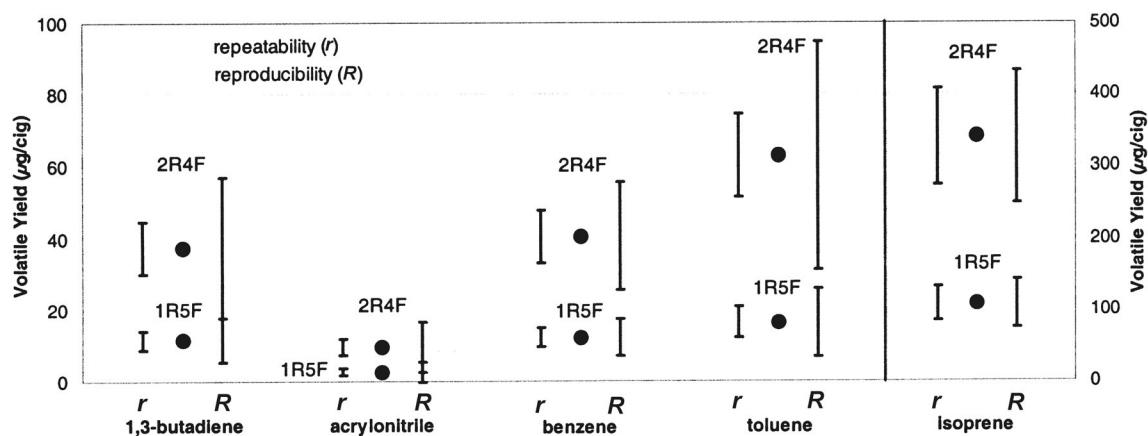
Figure 8. Summary of selected volatile yields and their variability
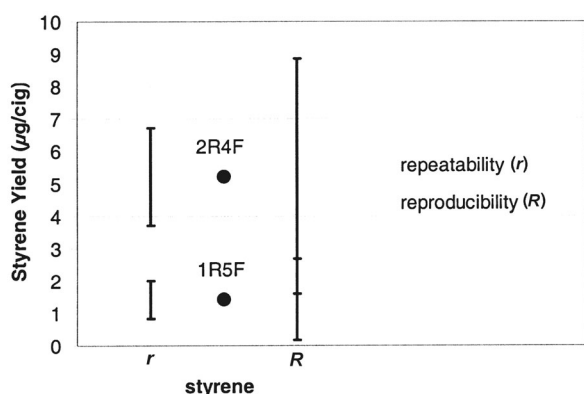
Figure 9. Summary of styrene yields and their variability

Fourteen laboratories measured selected volatiles by GC-MS with SIM and the other three laboratories applied GC-FID (flame ionisation detection) (laboratories 3, 11 and 17).

Other experimental factors such as choice and quality of calibration standards were not considered in this study.

The mean, maximum and minimum yields are shown graphically in Appendix 38. It is noteworthy that Laboratories 3 (direct trapping) and 19 found markedly more butadiene than the others. Laboratory 9 measured the highest yields for acrylonitrile. There is some indication that the relatively high 2R4F yield for Laboratory 17 is associated with a relatively high TPM yield.

The pattern of selected volatiles from Laboratory 19 was different to all the others (see Appendix 39)[b].

From the r and R data given in Figure 8 it can be seen that butadiene, acrylonitrile and toluene give variable data and it is not possible to always differentiate samples among the participating laboratories whereas benzene and isoprene are better differentiated.

It can be noted that the printing of benzene yields, one of the selected volatiles, is already mandated on Canadian cigarette packets (25) by Health Canada methods (5) and a rigorously standardised method would be beneficial.

Semi-volatiles methodology

Appendices 40 and 42 show a summary of the methodology employed to measure styrene, pyridine and quinoline in cigarette smoke by the contributing laboratories.

The semi-volatiles can trap to some extent on CFPs whilst a proportion will filter through and still need to be trapped by other means. The separation into "semi-volatiles" and "selected volatiles" is therefore somewhat arbitrary. Eleven laboratories trapped smoke by a combination of impingers and CFPs. Four laboratories smoked into one impinger and another 5 smoked into two traps. A further two laboratories had a trapping device consisting of three or even four liquid traps. The total volume of the impingers ranged from 20 mL to 50 mL. Three laboratories (numbers 5, 14 and 18) trapped smoke onto XAD-4 and CFP while one used acid impregnated CFPs.

Thirteen laboratories measured semi-volatiles by GC-MS coupled with SIM. Two laboratories (11 and 17) applied GC-FID.

Appendices 41 and 43 show graphically the mean, maximum and minimum yields obtained for the semi-volatiles. There is some indication that the relatively high 2R4F and 1R5F yields for Laboratory 6 are associated with relatively high TPM yields.

Laboratory 14 measured the highest yields for pyridine and styrene. Laboratory 3 sampled only styrene in the vapour phase. The pattern of semi-volatiles from Laboratory 19 was different to all others [b].

For styrene in Figure 9, the R values significantly overlapped and so it may not be possible to always differentiate samples among the participating laboratories.

For pyridine and quinoline in Figure 10, R values also overlapped and again it may not be possible to always differentiate samples among the participating laboratories.

Tobacco-specific nitrosamines (TSNAs) methodology

The methodology is summarised in Appendix 44. Six laboratories applied the more modern LC-MS-MS method without any sample clean up whereas seven laboratories carried out TSNA determinations according to the CORESTA recommended method (19) by GC-TEA and clean up. One of the latter laboratories provided two sets of data after collecting smoke on both linear and rotary

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[b] Subsequent to all the statistical analyses for this report, laboratory 19 identified errors in yield calculations for these analytes that would put their data in better line with data from other laboratories. However, these changes do not affect the overall conclusions made about this group of analytes in this report.
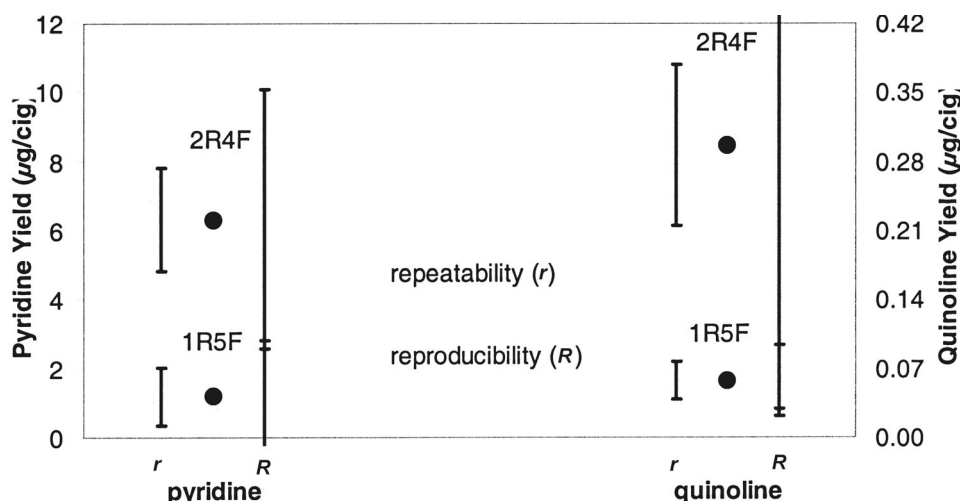
Figure 10. Summary of pyridine and quinoline yields and their variability
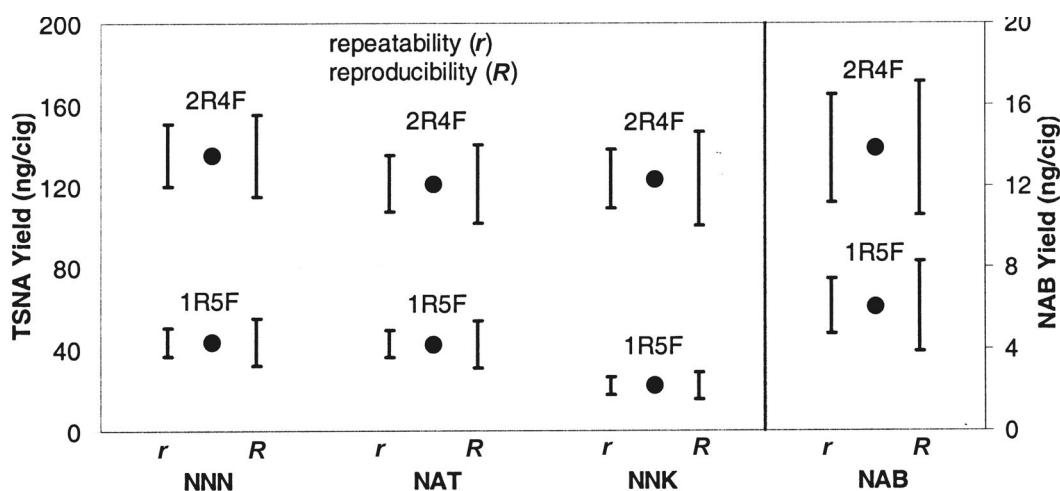
Figure 11. Summary of TSNA yields and their variability

smoking machines. There were no obvious differences from results obtained either with GC-TEA and LC-MS-MS.

Good differentiation was found between products among all participating laboratories with similar values for r and R as shown in Figure 11.

The mean, maximum and minimum yield data for the TSNAs are shown graphically in Appendix 45.

The mean yield data and reproducibility values R were obtained for the 2R4F cigarette as part of the collaborative study on TSNAs in 2005 where all participating laboratories used the same method. This is shown alongside mean yield and variability data (R) from this joint experiment for the 2R4F cigarette as given below:

Mean 2005	Mean 2006
NNN: 146.01 ng/cig ($R = 51.06$)	135.2 ng/cig ($R = 35.1$)
NAB: 16.60 ng/cig ($R = 9.16$)	13.8 ng/cig ($R = 5.8$)
NAT: 143.38 ng/cig ($R = 76.95$)	121.1 ng/cig ($R = 32.7$)
NNK: 141.39 ng/cig ($R = 73.44$)	123.7 ng/cig ($R = 40.8$)

Results are fairly comparable and demonstrate the value of standardisation in reducing data variability. Data variability

may even have improved since the collaborative study as many laboratories are measuring the TSNAs on a fairly regular basis. Even so, the data also demonstrates the relatively high level of variation obtained even using standardised methods and the high variability expected from other similar smoke analytes even after standardisation.

CONCLUSIONS

The collected data should be useful to individual laboratories for their internal method validation and accreditation purposes. As a means of continuous improvement, the data comparisons with others allow laboratories to identify the strengths and flaws or weaknesses in their current methods. As a general overview of the current situation, the preferred methodologies across a wide range of laboratories have been identified and demonstrate quite well the current worldwide expertise in this field.

For most analyses, the participating laboratories obtained quite different yields from the 1R5F and 2R4F cigarettes. However, the approach used to determine the current method performance was to investigate the level of statistical differentiation that could be achieved between

1R5F and 2R4F among laboratories when different laboratories were applying their different methodologies. It was felt that if the methods could not well differentiate these products then there were significant weaknesses in the application of at least some of these methods and in the future this could have wider regulatory implications. Even for the methods with the least among-laboratory variability there was still a need for rigorous standardisation and for properly conducted collaborative studies to obtain good estimates of repeatability within-laboratories and reproducibility among-laboratories.

Results indicate that "Hoffmann analyte" data are generally more variable both within- and among-laboratories than NFDPM; nicotine and carbon monoxide. Accordingly, tolerances around methods adopted for regulatory purposes will need to be proportionately higher.

It should be noted that ISO standardised B[a]P and the CORESTA recommended TSNA methods give some of the most reproducible results, showing the value of the standardisation process.

However, this data strongly suggests that the among-laboratory variability (R) is much higher for B[a]P and TSNAs than for NFDPM, nicotine and CO.

Based only on the two available one point in time studies, the indications are that tolerance values for the 9 mg 2R4F cigarette should be at least

▸ Mean $\pm$ 35% from the 2003 data and mean $\pm$ 45% from the current study for B[a]P, that is, mean $\pm R$.

▸ Mean ranging $\pm$ 35–55% from the 2005 data and mean $\pm$ 26–42% from the current study under the ISO smoking regime for the individual TSNAs, that is, mean $\pm R$.

Much work still needs to be carried out to take most of the various methods towards standardisation and CORESTA work is focusing on methods that are not currently differentiating well between reference cigarettes.

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Appendix 1. Study protocol

OBJECTIVE

The main objective of this study was to describe the variability of individual analyte yields obtained by various laboratories using various methodologies and attempt to model the variability as far as possible.

ANALYTES AND METHODS

The analytes to be included in the study were:

Table A1: Analytes investigated in the study

No.	Class and Analyte(s)	No.	Class and Analyte(s)
1	*Ammonia*	7	*Tobacco-specific N-nitrosamines*
			N-nitrosonornicotine (NNN)
2	*Aromatic amines*		4-(N-nitrosomethylamino)-1-(3-I)-1-butanone (NNK)
	1- aminonaphthalene		N-nitrosoanatabine (NAT)
	2- aminonaphthalene		N-nitrosoanabasine (NAB)
	3- aminobiphenyl	8	*Semi-volatiles*
	4- aminobiphenyl		Pyridine
3	*Benzo[a]pyrene*		Quinoline
			Styrene
4	*Volatile carbonyls*	9	*Phenolic compounds*
	Formaldehyde		Hydroquinone
	Acetaldehyde		Resorcinol
	Acetone		Catechol
	Acrolein		Phenol
	Propionaldehyde		m+p-Cresol
	Crotonaldehyde		o-Cresol
	MEK (methyl ethyl ketone)	10	*Selected volatiles*
	Butyraldehyde		1,3 Butadiene
5	*Hydrogen cyanide*		Isoprene
			Acrylonitrile
6	*NO*		Benzene
			Toluene

The method(s) applied in determining the yields of the analytes listed in the above table was the in-house method currently being used by each participating laboratory. It was not required that each participating laboratory make determinations for all of the analytes listed, but rather as many as are possible given the constraints of the laboratory.

Since no single test method was required to be followed for any of the analyte determinations, the specifics of each method utilized by a laboratory were documented in the laboratory report.

STUDY PROTOCOL

Cigarette samples smoked were Kentucky reference 2R4F (A) and Kentucky reference 1R5F (B).

Smoking plans

Five replicates for each test sample should be generated in 3 independent experiments. Products should be smoked randomly and smoking plans allowing this should be used.

Fully orthogonal random smoking plans cannot be generated, but random smoking plans may be obtained with the help of a random number generator (random permutation of 5 replicates of 2 brands).

Each experiment must be smoked on a different day, separated by minimum of one week, in order to incorporate day-to-day variation into the resulting data. If it is not possible to generate the 10 samples required for each experiment in a single day, the sample generation can be carried over to consecutive days.

Table A2. Example for rotary smoking machines

Experiment No. 1	Experiment No. 2	Experiment No. 3
A	B	B
B	B	A
B	A	A
A	B	A
B	A	B
A	B	A
A	A	B
B	A	A
A	B	B
B	A	B

In each experiment, 10 ports will be used and 10 "blank" ports will remain unused, Table A3:

Table A3. Example for 20 port linear smoking machines

Exp. No.	Port									
	1	2	3	4	5	6	7	8	9	10
1		B	A		A			B		
2		A		B		A	A		A	
3	B	A	A	B			A			B

Exp. No.	11	12	13	14	15	16	17	18	19	20
1	A	B	A			B	A		B	
2	B			B				B	A	B
3	B	A				A				B

In each experiment, 10 ports will be used and 6 "blank" ports will remain unused, Table A4:

Table A4. Example for 16 port linear smoking machines

Exp. No.	1	2	3	4	5	6	7	8
1	B	A		B	A	A		B
2		B	A	B	A	A	B	
3	B	B	A	B	B		A	

Exp. No.	9	10	11	12	13	14	15	16
1		B		B	A	A		
2	A	B		A				B
3	A			B	A			A

In each experiment, all 10 ports will be used, Table A5:

Table A5. Example for 10 port linear smoking machines

Exp. No	1	2	3	4	5	6	7	8	9	10
1	B	B	B	B	A	A	A	B	A	A
2	A	A	A	B	B	A	A	B	B	B
3	A	A	B	A	B	B	A	A	B	B

In each experiment, the 10 samples will be split into two runs of 5 samples, Table A6:

Table A6. Example for 5 port linear smoking machines

Exp. No.	Run No.	1	2	3	4	5
1	1	B	B	B	B	A
	2	A	A	B	A	A
2	1	A	A	A	B	B
	2	A	A	B	B	B
3	1	A	A	B	A	B
	2	B	A	A	B	B

DATA SUBMISSION AND ANALYSIS

A template was used for data submission and test methods used for each analyte or groups were submitted along with the data. The data was analysed independently by 3 statisticians to ensure that as much data interpretation was made as possible. The laboratories mainly performed the study from November 2005 to July 2006 and results were sent to the data coordinators and then on to the statisticians by August 2006.

Appendix 2. Summary of all received mean data on Kentucky Reference 2R4F cigarettes

Table A7. Summary of all received mean data on 2R4F cigarettes in laboratories 1–19

Smoke analyte	1	2	3	4	5	6A	6B	7	8	9	10	11	12	13	14	15	16	17	18	19
Ammonia	10.1	ND	25.4	10.1	10.6	12.8		10.3	ND	ND	ND	10.6	10.2	ND	6.6	ND	12.0	9.1	10.2	ND
1-Naphthyl amine	12.8	ND	7.0	13.6	14.9	10.3		9.0	ND	ND	ND	ND	11.0	11.3	ND	ND	16.3	12.0	18.1	8.1
2-Naphthyl amine	9.8	ND	7.4	9,6	10.1	7.8		5.7	ND	6.1	ND	ND	8.9	7.6	ND	ND	10.5	8.1	13.2	4.7
3-Amino biphenyl	2.2	ND	1.6	2.1	2.2	2.1		2.0	ND	ND	ND	ND	2.0	2.3	ND	ND	2.3	2.0	3.9	1.6
4-Amino biphenyl	1.7	ND	1.3	1.5	1.5	1.3		1.3	ND	1.1	ND	ND	1.5	1.1	ND	ND	1.9	1.4	3.0	1.0
B[a]P	6.0	ND	7.5	6.2	7.3	5.6		5.9	ND	7.2	8.5	7.0	8.3	8.9	7.5	7.7	6.4	6.2	6.4	8.5
Formaldehyde	25.3	20.2	24.2	23.3	29.8	8.6		22.2	23.3	18.2	13.4	14.5	24.1	ND	22.3	ND	23.3	17.1	18.8	21.3
Acetaldehyde	629.8	435.9	481.8	628.8	546.4	655.1		655.1	732.8	546.9	546.2	562.0	649.8	ND	ND	ND	452.9	673.0	616.9	585.7
Acrolein	60.6	48.9	55.2	61.7	56.9	54.5		60.4	61.3	52.1	49.2	4.9	60.7	ND	ND	ND	50.1	53.3	46.6	37.9
Acetone	297.2	238.3	227.1	242.3	292.6	261.9		296.1	300.6	ND	251.8	244.9	267.0	ND	ND	ND	265.3	329.9	284.8	256.6
MEK	76.5	57.8	45.8	61.6	69.2	79.5		92.7	94.2	ND	64.9	64.6	114.9	ND	ND	ND	63.3	76.7	56.9	69.1
Butyraldehyde	31.3	28.3	23.7	30.1	32.0	29.8		30.5	34.2	ND	29.8	63.3	38.1	ND	ND	ND	30.0	27.3	32.2	32.2
Propionaldehyde	46.0	39.8	42.5	44.7	48.0	56.6		53.9	60.9	45.0	44.3	41.3	53.0	ND	ND	ND	43.7	51.2	72.2	41.2
Crotonaldehyde	14.6	18.6	11.1	23.8	13.9	12.2		17.0	20.1	ND	17.5	47.7	17.1	ND	ND	ND	11.9	11.2	20.2	12.7
HCN	101.9	94.0	94.8	119.2	113.3	133.0		122.5	ND	82.0	164.1	53.1	123.4	74.3	149.3	ND	106.9	ND	92.0	ND
NO	218.6	236.6	225.1	248.1	195.2	181.7		159.7	ND	252.0	ND	228.7	237.3	ND	190.9	ND	233.8	ND	228.2	ND
Phenol	11.0	7.7	5.1	10.2	6.4	5.8		7.2	8.0	6.8	7.5	5.7	10.0	6.1	8.7	ND	7.8	7.9	9.0	7.2
o-Cresol	3.1	2.3	1.3	3.2	2.2	1.8		2.2	2.1	ND	2.0	1.2	2.1	2.7	2.1	ND	2.3	2.9	3.0	1.6
m/p-Cresol	8.1	6.4	3.5	8.2	6.6	5.8		5.9	5.7	ND	5.3	3.6	6.0	7.5	5.0	ND	6,4	7.5	8.1	4.8
Hydroquinone	33.9	33.8	28.6	33.5	32.6	31.9		31.0	30.9	ND	36.0	30.2	43.7	26.5	34.2	ND	30.1	32.5	31.4	22.2
Catechol	44.4	43.7	34.1	40.5	41.1	41.2		40.0	40.2		39.5	36.1	41.3	44.4	38.8	ND	39.7	40.6	44.3	33.8
Resorchinol	0.7	2.7	0.7	1.0	0.8	0.7		0.7	0.7		NN	1.3	0.8	0.6	2.0	ND	0.6	0.6	0.7	2.7
1,3-Butadiene	32.1	24.6	68.7	38.7	32.5	ND	30.1	35.2	ND		22.4	ND	36.7	ND	ND	ND	37.0	39.5	39.4	61.9
Isoprene	342.0	364.4	304.8	404.3	276.7	303.0	312.5	412.7	ND		284.4	381.1	368.5	293.3	ND	ND	316.5	449.5	315.7	300.0
Acrylonitrile	7.7	7.5	16.1	7.0	8.8	10.1	10.2	8.6	ND		8.4	14.0	9.2	5.6	ND	ND	8.1	9.5	12.0	4.9
Benzene	34.7	39.2	43.3	35.0	46.5	38.0	47.2	46.6	ND		45.3	54.9	46.4	40.8	ND	ND	35.7	45.3	47.4	12.9
Toluene	57.9	58.2	66.7	44.0	66.3	61.9		79.9	ND		82.8	85.4	76.9	69.1	ND	ND	56.9	73.4	70.8	5.6
NNN	137.6	ND	154.3	126.1	120.0	119.3	119.1	142.2	ND		144.9	ND	143.8	ND	ND	ND	144.7	127.9	133.8	144-8
NAT	117.9	ND	136.2	119.5	112.7	112.3	110.0	118.6	ND		115.0	ND	125.6	ND	ND	ND	138.2	107.4	119.6	141.1
NNK	119.9	ND	151.6	118.9	109.7	115.0	112.2	131.2	ND		140.9	ND	122.7	ND	ND	ND	134.3	103.0	117.5	135.8
NAB	13.1	ND	16.0	14.6	13.5	12.2	12.4	12.7	ND		14.2	ND	13.5	ND	ND	ND	16.4	12.2	11.2	17.2
Pyridine	5.0	ND	ND	5.8	5.7	9.7		5.2	4.2		6.2	5.3	5.8	6.0	10.6	ND	8.7	6.8	7.8	1.1
Quinoline	1.18	ND	ND	0.24	0.35	0.25		0.20	0.21		0.26	0.20	0.3	0.21	0.23	ND	0.23	ND	0.33	0.85
Styrene	3.8	ND	3.4	3.7	6.6	n/p		5.8	n/p		5.9	2.4	6.3	3.3	10.6	ND	6.8	5.3	5.0	0.7

Note: Each mean value in this table is the simple average of all individual replicates across 3 experiments

Table A8. Summary of all received mean data on 1R5F cigarettes in laboratories 1–19

Smoke analyte	1	2	3	4	5	6A	6B	7	8	9	10	11	12	13	14	15	16	17	18	19
Ammonia	2.7	ND	5.5	2.8	2.8	2.2		3.2	ND	ND	ND	2.9	4.9	ND	1.4	ND	3.3	2.0	3.0	ND
1-Naphthyl amine	3.1	ND	0.3	4.5	6.9	3.4		2.9	ND	ND	ND	ND	2.5	5.4	ND	ND	4.1	3.2	2.7	4.9
2-Naphthyl amine	2.3	ND	0.7	3.1	<3.4	2.8		1.9	ND	1.7	ND	ND	2.0	4.2	ND	ND	2.7	2.0	2.8	2.6
3-Amino biphenyl	0.7	ND	0.2	0.8	<1.1	0.8		0.8	ND	ND	ND	ND	0.9	1.5	ND	ND	0.7	0.6	1.4	1.6
4-Amino biphenyl	0.5	ND	0.2	0.6	0.6	0.6		0.5	ND	0.3	ND	ND	0.7	1.6	ND	ND	0.6	0.4	0.9	0.7
B[a]P	1.1	ND	1.7	1.3	2.4	1.0		1.3	ND	1.7	1.8	1.8	1.6	1.8	1.6	1.5	1.2	1.8	1.4	2.6
Formaldehyde	3.6	3.7	4.2	3.4	8.8	1.5		2.7	3.8	2.8	2.1	17.7	3.0	ND	5.1	ND	4.5	1.8	5.0	2.3
Acetaldehyde	147.2	110.5	136.3	161.7	154.5	176.7		164.4	189.5	160.5	148.3	167.3	130.8	ND	ND	ND	131.3	150.8	174.4	168.4
Acrolein	10.7	9.6	12.7	12.9	11.6	11.2		12.0	11.9	12.5	9.7	3.1	11.2	ND	ND	ND	11.8	7.7	6.2	6.9
Acetone	89.5	70.4	69.1	65.3	94.7	68.8		82.8	68.7	ND	64.8	68.5	62.4	ND	ND	ND	87.5	81.7	95.2	83.4
MEK	17.8	15.6	13.0	14.7	18.9	19.7		22.6	22.3	ND	14.4	12.9	44.7	ND	ND	ND	17.2	15.7	15.1	22.7
Butyraldehyde	8.3	8.5	7.5	8.8	9.4	8.1		8.6	10.0	ND	8.0	20.1	6.2	ND	ND	ND	9.8	6.9	10.5	11.3
Propionaldehyde	11.6	10.6	13.1	11.6	14.1	13.9		14.5	17.0	14.4	11.3	12.5	9.5	ND	ND	ND	13.3	12.2	23.2	12.5
Crotonaldehyde	2.0	3.7	2.2	4.3	1.9	0.6		2.2	3.1	ND	3.2	2.3	3.6	ND	ND	ND	2.3	1.3	4.4	2.4
HCN	19.3	18.4	17.5	14.3	21.5	20.7		21.5	ND	17.5	39.8	13.8	19.4	5.7	36.3	ND	20.1	ND	14.1	ND
NO	126.0	123.0	94.1	113.8	85.7	89.6		62.7	ND	122.9	ND	103.2	78.5	ND	103.3	ND	114.7	ND	100.2	ND
Phenol	1.4	0.7	0.6	1.4	0.8	0.6		1.0	0.9	1.2	1.6	0.7	1.3	0.5	0.9	ND	0.9	0.6	0.3	1.1
o-Cresol	0.5	0.2	0.3	0.5	0.4	0.2		0.5	0.4	ND	0.5	0.2	0.7	0.3	0.2	ND	0.3	0.4	0.2	0.1
m/p-Cresol	1.4	0.5	0.7	1.4	1.1	0.8		1.2	0.7	ND	1.3	0.6	0.9	1.0	0.6	ND	1.0	1.0	1.0	0.6
Hydroquinone	8.6	9.2	9.0	8.8	8.4	5.9		8.3	7.1	ND	12.9	7.9	6.1	6.1	7.9	ND	7.7	6.8	5.0	5.0
Catechol	0.2	9.1	8.2	8.7	8.5	8.1		9.2	7.9	8.6	11.6	7.7	8.1	8.2	7.1	ND	8.1	7.2	6.8	6.0
Resorchinol	8.3	0.6	0.2	0.2	0.2	0.1		<LOQ	0.1	ND	ND	0.3	<0.1	0.1	<LOQ 0.5	ND	0.2	0.1	0.1	0.5
1,3-Butadiene	96.0	7.9	22.0	11.5	9.5	ND	8.8	11.1	ND	13.5	7.2	ND	11.0	ND	ND	ND	11.3	11.0	10.5	18.1
Isoprene	1.9	123.2	103.6	132.0	102.2	89.0	107.2	131.7	n/p	137.4	80.2	121.5	102.9	87.3	ND	ND	102.6	134.8	113.8	92.0
Acrylonitrile	9.0	1.6	3.7	1.9	2.5	2.4	3.3	1.9	n/p	8.5	2.3	3.3	3.1	1.8	ND	ND	2.0	1.7	2.0	1.1
Benzene	13.0	12.2	13.2	11.7	14.5	9.6	14.5	14.5	n/p	17.9	13.2	15.0	12.9	12.5	ND	ND	11.2	12.8	13.5	3.6
Toluene	41.0	14.3	17.3	12.2	23.8	14.3		20.9	n/p	24.4	21.3	27.4	16.0	22.2	ND	ND	16.8	19.7	20.6	1.4
NNN	39.1	ND	52.9	43.4	43.9	32.2	35.5	50.5	ND	46.0	48.0	ND	53.4	ND	ND	ND	48.4	37.9	36.4	41.1
NAT	20.2	ND	52.0	45.7	46.1	34.2	37.5	48.6	ND	ND	39.0	ND	43.4	ND	ND	ND	51.7	35.7	31.9	47.0
NNK	5.1	ND	30.2	20.4	22.5	17.5	18.9	25.5	ND	21.2	23.8	ND	25.8	ND	ND	ND	24.0	17.1	17.5	25.4
NAB	0.8	ND	7.7	6.5	6.1	<LOQ 10	<LOQ 10	6.2	ND	ND	5.8	ND	<LOQ	ND	ND	ND	7.5	4.8	3.6	7.2
Pyridine	0.04	ND	ND	0.6	0.9	1.6		0.6	0.6	ND	1.2	1.6	0.6	1.1	4.3	ND	1.8	1.6	0.9	0.2
Quinoline	0.6	ND	ND	0.06	0.06	0.05		0.07	0.04	ND	0.06	<LOQ	0.04	0.04	<LOQ 0.1	ND	0.05	ND	0.06	0.12
Styrene		ND	1.1	0.8	1.6	n/p		1.3	n/p	1.7	1.5	1.3	<2.0	1.4	1.9	ND	2.3	3.2	1.2	0.1

Note: Each mean value in this table is the simple average of all individual replicates across 3 experiments

Appendix 4. Summary of all received mean data on Kentucky Reference 2R4F cigarettes

Table A9. Summary of mean data, standard deviation (SD), coefficient of variation % (CoV), maximum yield (Max), minimum yield (Min) and Max/Min on 2R4F cigarettes The mean and SD were calculated directly from the individual laboratory mean values shown in Appendix 2.

Smoke analyte	Mean	SD	CoV%	Max	Min	Min/Max	Data Sets
Catachol	40.1	3.2	7.9	44.4	33.8	1.3	18
NNN	135.2	11.3	8.3	154.3	119.1	1.3	14
NAT	121.1	11.0	9.1	141.1	107.4	1.3	13
Acetone	270.4	28.7	10.6	329.9	227.1	1.5	15
NNK	123.7	13.4	10.6	151.6	103.0	1.5	14
NO	218.1	27.8	12.7	252.0	159.7	1.6	13
NAB	13.8	1.8	13.2	17.2	11.2	1.5	13
Hydroquinone	31.9	4.4	13.9	43.7	22.2	2.0	17
Acetaldehyde	587.4	83.2	14.2	732.8	435.9	1.7	16
B[a]P	7.1	1.0	14.3	8.9	5.6	1.6	17
Isoprene	341.2	50.5	14.8	449.5	276.7	1.6	17
Propionaldehyde	49.0	8,7	17.7	72.2	39.8	1.8	16
Phenol	7.7	1.6	21.3	11.0	5.1	2.2	18
Benzene	41.8	9.4	22.5	54.9	12.9	4.2	17
m/p-Cresol	6.2	1.4	23.3	8.2	3.5	2.3	17
MEK	72.5	17.5	24.1	114.9	45.8	2.5	15
Formaldehyde	20.6	5.1	24.8	29.8	8.6	3.5	17
o-Cresol	2.2	0.6	26.0	3.2	1.2	2.5	17

Table A9 (*cont.*)

Smoke analyte	Mean	SD	CoV%	Max	Min	Min/Max	Data Sets
HCN	108.2	28.7	26.6	164.1	53.1	3.1	15
3-Amino biphenyl	2.2	0.6	26.6	3.9	1.6	2.5	12
2-Naphthyl amine	8.4	2.3	27.0	13.2	4.7	2.8	13
Acrolein	50.8	13.9	27.3	61.7	4.9	12.6	16
Butyraldehyde	32.8	9.0	27.4	63.3	23.7	2.7	15
1-Naphthyl amine	12.0	3.3	27.6	18.1	7.0	2.6	12
Toluene	65.0	19.6	30.1	85.4	5.6	15.2	16
1.3-Butadiene	38.2	12.7	33.2	68.7	22.4	3.1	14
4-Amino Biphenyl	1.5	0.5	33.7	3.0	1.0	3.0	13
Pyridine	6.3	2.3	36.9	10.6	1.1	10.0	15
Ammonia	11.5	4.6	40.3	25.4	6.6	3.9	12
Acrylonitrile	10.1	4.5	44.7	23.9	4.9	4.9	17
Styrene	5.0	2.3	46.1	10.6	0.7	14.4	15
Crotonaldehyde	18.0	9.1	50.4	47.7	11.1	4.3	15
Quinoline	0.3	0.2	58.8	0.9	0.2	4.7	14
Resorcinol	1.1	0.7	68.6	2.7	0.6	4.5	16

Appendix 5. Summary of all received mean data on Kentucky Reference 1R5F cigarettes

Table A10. Summary of mean data, standard deviation (SD), coefficient of variation % (CoV), maximum yield (Max), minimum yield (Min) and Max/Min on 1R5F cigarettes The mean and SD were calculated directly from the individual laboratory mean values shown in Appendix 3.

Smoke analyte	Mean	SD	CoV%	Max	Min	Min/Max	Data Sets
Acetaldehyde	158.2	17.4	11.0	189.5	130.8	1.4	14
Catechol	8.1	1.2	15.1	11.6	6.0	1.9	16
Acetone	76.4	11.6	15.2	95.2	62.4	1.5	13
NNN	43.8	6.9	15.7	53.4	32.2	1.7	13
NAT	42.7	6.9	16.1	52.0	31.9	1.6	12
Isoprene	109.2	18.6	17.0	137.4	80.2	1.7	15
NNK	22.3	4.0	17.8	30.2	17.1	1.8	13
NO	97.2	17.5	18.0	122.9	62.7	2.0	11
NAB	6.2	1.3	21.5	7.7	3.6	2.1	9
Propionaldehyde	13.8	3.2	23.4	23.2	9.5	2.4	14
Benzene	12.7	3.1	24.8	17.9	3.6	5.0	15
B[a]P	1.6	0.4	25.2	2.6	1.0	2.6	16
Hydrtoquinone	7.5	2.0	26.1	12.9	5.0	2.6	15
m/p-Cresol	0.9	0.3	28.5	1.4	0.6	2.5	15
Acrolein	10.1	3.0	29.4	12.9	3.1	4.1	14
1,3-Butadiene	12.1	4.1	33.8	22.0	7.2	3.0	12
Toluene	18.5	6.4	34.8	27.4	1.4	19.4	14
Butyraldehyde	9.6	3.5	36.0	20.1	6.2	3.2	13
2-Naphthyl amine	2.4	0.9	37.3	4.2	0.7	5.9	11
Phenol	0.9	0.3	37.5	1.6	0.3	4.7	16
Quinoline	0.1	0.0	38.2	0.1	0.0	3.0	11
Ammonia	3.1	1.2	39.0	5.5	1.4	4.0	11
o-Cresol	0.3	0.1	42.0	0.7	0.1	4.6	15
Crotonaldehyde	2.6	1.1	42.1	4.4	0.6	7.3	13
MEK	19.5	8.4	42.8	44.7	12.9	3.5	13
HCN	20.2	9.1	45.0	39.8	5.7	7.0	13
1-Naphthylamine	3.7	1.7	47.1	6.9	0.3	23.0	11
3-Amino biphenyl	0.9	0.4	47.2	1.6	0.2	7.9	10
Styrene	1.5	0.7	50.0	3.2	0.1	35.6	13
4-Amino biphenyl	0.6	0.4	56.8	1.6	0.2	8.1	12
Resorcinol	0.2	0.1	61.3	0.5	0.1	5.3	11
Acrylonitrile	2.8	1.7	63.1	8.5	1.1	7.5	15
Pyridine	1.2	1.0	80.6	4.3	0.2	22.6	14
Formaldehyde	4.6	4.1	88.7	17.7	1.5	11.8	15

Appendix 6. Statistical analysis – removed data

Table A11. Statistical analysis – removed data (LOQ = limit of quantification)

Smoke analyte	Lab no.	Data removed from statistical analyses	Lab no.	Data excluded in comparison of 2R4F vs 1R5F within a laboratory
Quinoline	10	Removed - LOQ related	6B	
			14	
Acrylonitrile	10	Removed - LOQ related		
Toluene	6B	1 value removed - transcription error?		
Toluene	5	Removed - LOQ related		Some or all values were below the LOQ giving unbalanced comparisons between 1R5F and 2R4F cigarettes
2-Amino naphthalene	5	Removed - LOQ related		
3-Amino biphenyl	13	Less than 10 data points	5	
Resorcinol			7	
			12	Data was only excluded for differentiations between 2R4F and 1R5F in section 4.3.4
			14	
o-Cresol			7	
Styrene			12	
NAB			6A	
			6B	
			12	

Appendix 7. Statistical analysis – Estimation of values of within-laboratory (*r*) and among laboratory (*R*) variability

The statistical analysis (as used in the Experimental section) is based in principle on the recommended International Standard ISO 5725 method (22) as described in part 2 and part 6, section 4 of the standard.

The following mathematical equations were used to calculate the values of *r* and *R* for sample A. Values for sample B are obtained in a similar way.

$$r = 2 \{2[SD(r_A)^2 / 5]\}^{\frac{1}{2}}$$
$$R = 2 \{2[SD(L_A)^2 + (SD(r_A)^2 / 5)]\}^{\frac{1}{2}}$$
$$\%CoV\ (r) = 100\ r\ /\ \text{Mean A}$$
$$\%CoV\ (R) = 100\ R\ /\ \text{Mean A}$$

where

- SD(L_A) and SD(L_B) are the standard deviations among laboratories for samples A (2R4F) and B (1R5F)
- SD(r_A) and SD(r_B) are the standard deviations between replicates, pooled over laboratories and experiments. The denominator 5 in the equation is included to define *r* for differences under within-laboratory variability conditions between the average of 5 replicates for each analyte.
- Mean A-B is the mean difference between samples A (2R4F) and B (1R5F), averaged across laboratories.
- CoV is the coefficient of variation
- Sample A = 2R4F Kentucky reference cigarette
- Sample B = 1R5F Kentucky reference cigarette

The statistical analysis (as used in the Individual Methodology discussion section) is again based in principle on the recommend International Standard ISO method (22) but in this case the variation among all 15 replicates from each laboratory was used to define the 'within lab' variance.

$$s_r^2 = \frac{\sum_{i=1}^{p} (n_i - 1)\, s_i^2}{\sum_{i=1}^{p} (n_i - 1)}$$

is the within-laboratory variance for an analyte,

where

p = total number of laboratories whose data is being used,
n_i = the number of data points reported for lab *i* (generally 15, *replicates pooled for all experiments*),
s_i = the standard deviation among replicates and experiments for lab *i*,
$s_R^2 = s_L^2 + s_r^2$ is the among-laboratory variability variance for an analyte,

$$s_R^2 = \frac{s_d^2 - s_r^2}{\bar{n}} \quad \text{where} \quad s_d^2 = \frac{1}{p-1} \sum_{i=1}^{p} n_i \left(\bar{y}_i - \bar{\bar{y}}\right)^2$$

$$\text{and} \quad \bar{n} = \frac{1}{p-1} \cdot \sum_{i=1}^{p} n_i - \frac{\sum_{i=1}^{p} n_i^2}{\sum_{i=1}^{p} n_i} \quad ,$$

$\bar{y}_i$ = is the mean analyte yield for laboratory *i* and

$\bar{\bar{y}}_i$ = the analyte yield grand mean over all laboratories and replicates,

$$r = 2\ (2 \times S_r^2)^{1/2},$$
$$R = 2\ (2 \times S_R^2)^{\frac{1}{2}}.$$

179

Table A12. Within-laboratory (*r*) and among laboratory (*R*) variability values

Smoke analyte	No. of labs	Mean 2R4F	r 2R4F	R 2R4F	Mean 1R5F	r 1R5F	R 1R5F
Ammonia	11	11.58	0.94	13.85	3.06	0.32	3.51
Formaldehyde	16	20.96	2.46	14.76	3.64	0.68	5.20
Acetaldehyde	15	589.1	41.82	250.4	153.7	20.9	63.2
Acetone	14	272.3	20.48	86.74	77.46	10.28	38.53
Acrolein	15	53.90	4.67	21.24	10.56	1.72	6.49
Propionaldehyde	15	49.53	3.60	25.50	13.50	1.84	9.63
Crotonaldehyde	14	15.84	1.85	11.62	2.64	0.50	3.19
MEK	14	73.08	5.95	51.66	19.59	2.66	22.71
Butyraldehyde	14	30.67	2.43	10.09	8.70	1.16	4.47
HCN	14	112.19	12.24	73.24	22.42	3.57	25.24
NO	12	217.26	11.87	83.67	101.21	8.87	58.41
Pyridine	14	6.32	0.70	6.86	1.19	0.28	3.00
Quinoline	11	0.31	0.04	0.53	0.06	0.01	0.07
Hydroquinone	16	32.06	1.67	13.41	7.62	0.70	5.54
Resorcinol	12	1.03	0.10	2.23	0.20	0.08	0.45
Catechol	17	40.33	2.31	9.74	8.24	0.66	3.55
Phenol	17	7.78	0.69	4.77	0.93	0.20	1.04
m/p-Cresol	16	6.30	0.53	3.84	0.94	0.15	0.84
o-Cresiol	16	2.30	0.20	1.57	0.35	0.05	0.42
1,3-Butadiene	14	36.17	3.67	36.23	11.55	1.13	11.67
Isoprene	16	338.57	32.75	154.10	108.48	10.70	56.28
Acrylonitrile	15	9.94	0.90	13.44	2.62	0.35	5.08
Benzene	16	41.02	3.49	26.47	12.29	1.26	9.07
Toluene	14	63.46	4.99	58.07	18.73	2.14	16.66
Styrene	13	5.12	0.73	6.75	1.42	0.28	2.23
1-Naphthyl amine	12	12.04	1.10	9.75	3.66	0.32	4.76
2-Naphthyl amine	12	8.31	0.70	6.66	2.40	0.24	2.41
3-Amino biphenyl	10	2.16	0.22	1.90	0.84	0.13	1.12
4-Amino biphenyl	13	1.50	0.31	1.53	0.63	0.14	0.98
Benzo[*a*]pyrene	16	7.12	0.55	3.23	1.60	0.27	1.36
NNN	14	135.46	7.20	35.12	43.84	3.34	20.20
NAT	13	121.36	7.74	32.83	42.75	3.32	20.17
NAB	10	14.19	1.44	5.90	6.10	0.68	3.89
NNK	14	123.95	6.22	40.86	22.20	2.06	11.65

Appendix 8. Within-laboratory variability (*r*)
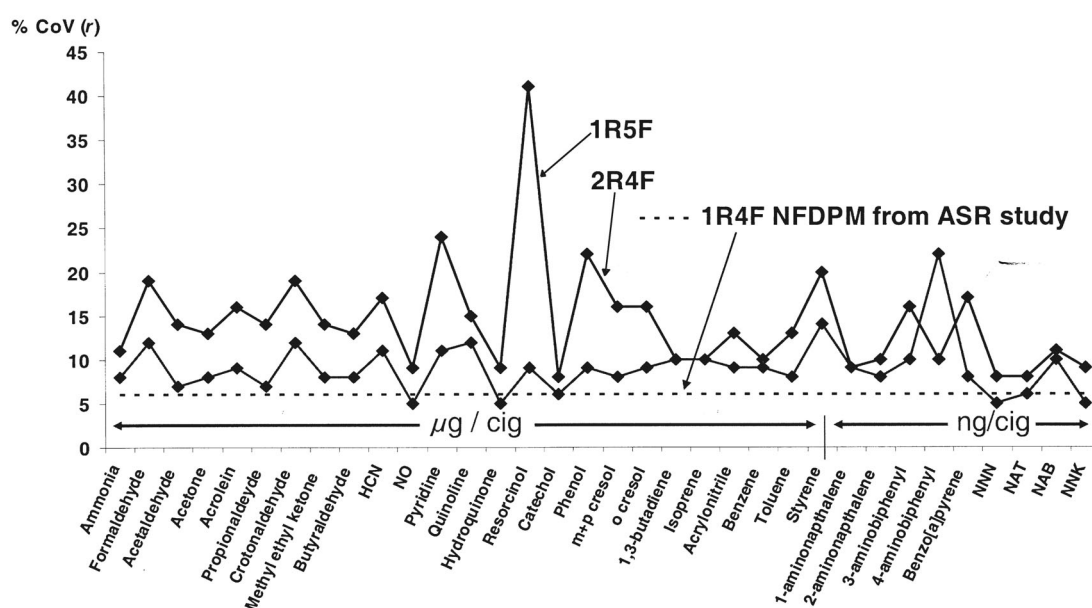
Figure A1. Within-laboratory variability (*r*) for various analyte yields in 1R5F and 2R4F cigarettes (where ASR = study on alternative smoking regimes (23); CoV = coefficient of variation

Appendix 9. Among-laboratory variability (R)
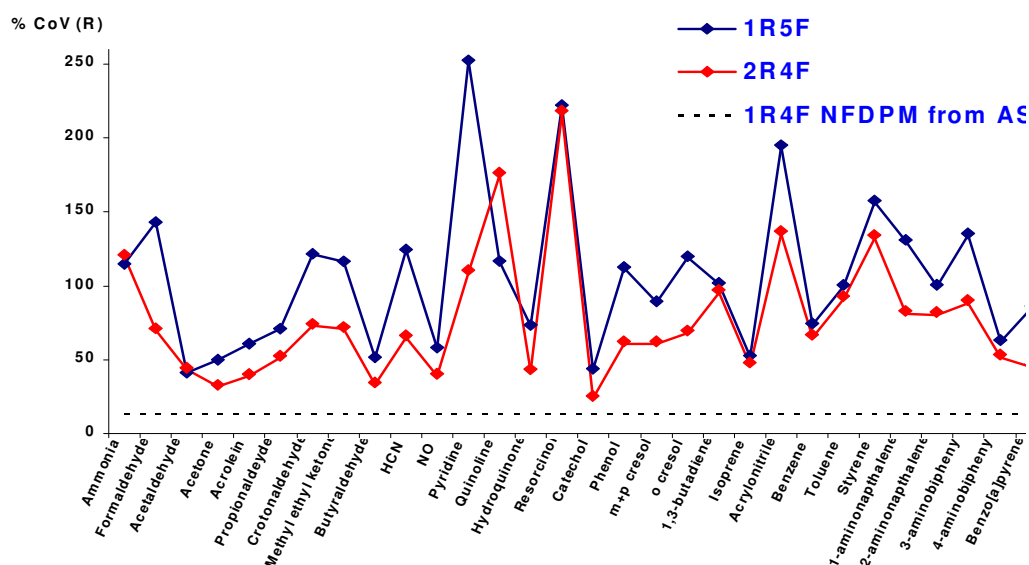
Figure A2. Among-laboratory variability (R) for various analyte yields in 1R5F and 2R4F cigarettes (where ASR = study on alternative smoking regimes (23); CoV = coefficient of variation)

Appendix 10. Normalised analyte variability among laboratories

%CoV (SD(b) = {100 × {SD(L)^2 +[SD(r)^2/ 5]}^{½}} / Mean

where

▸ D(L) is the Standard Deviation among laboratories (this includes the additional variability between experiments)

▸ SD(r) is the Standard Deviation among replicates, pooled over laboratories and experiments
▸ Mean is the average yield of each analyte across laboratories.
▸ SD(b) can be referred to as the Standard Deviation among laboratories for the average of five replicates.

Indication of expected magnitude of NFDPM variability for 2R4F cigarette (Mean yields on *x*-axis are given in µg/cigarette for measured analytes compared to 9 mg/cigarette for NFDPM)
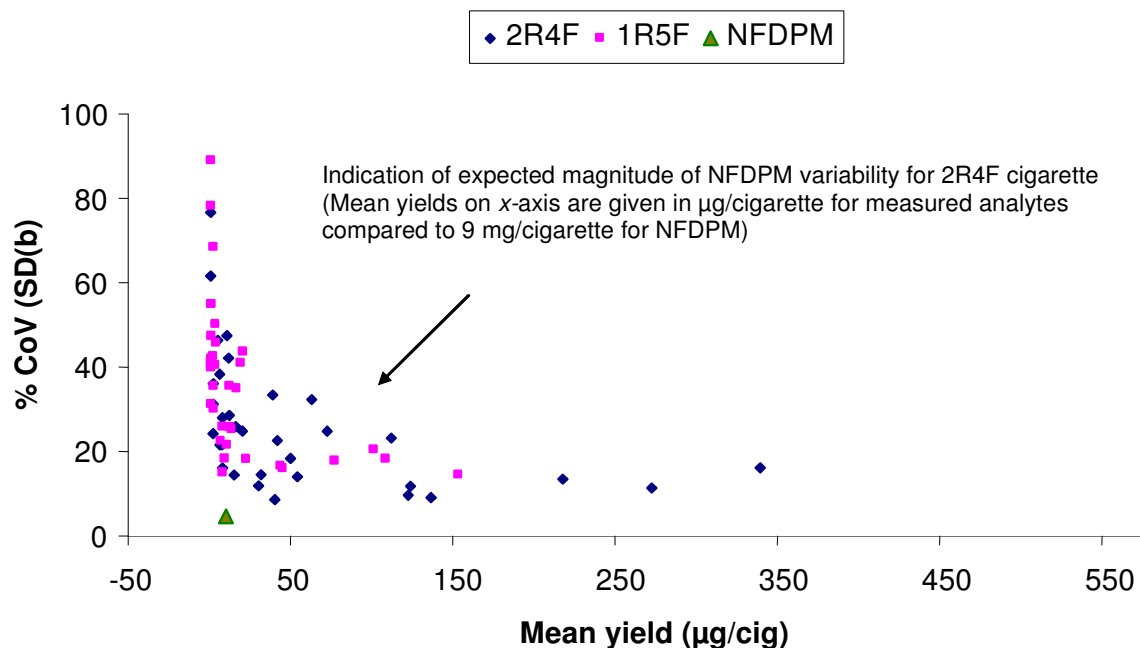
Figure A3. Normalised analyte variability among laboratories (mean yields on *x*-axis are given in µg/cig for measured analytes compared to 9 mg/cig for NFDPM)

181

A graphical summary of the extent to which each analyte was able to differentiate between samples A and B (2R4F and 1R5F) is given in Appendix 12. In this plot, the vertical axis defines the smallest difference between the mean yields for samples A and B that can be distinguished (with 95% confidence) when each sample has been tested by separate laboratories. Unavoidably, in order to put the different analytes on a common scale so that they can be directly compared, the precise scale of measurement is rather complicated. For ease of understanding concerning what each point indicates in the context of method standardisation:

‣ Points on the vertical axis which are ≥ 100 indicate that for the corresponding analytes it would not be possible to distinguish (with 95% confidence) between the samples A and B. For these analytes there would appear to be the greatest need to further standardise the laboratory methods.

‣ Conversely, for analytes whose points on the plot are < 100, the samples A and B would be distinguished (with 95% confidence). However, it is suggested that further work is still needed to standardise the laboratory methods for those analytes positioned close to 100; perhaps those above 75.

The mathematical equation below details how each value (one for each analyte) was calculated for the graphical plot in Appendix 12.

$$\%CoV\ [R(A\text{-}B)] = 100 \times R(A\text{-}B) / Mean(A\text{-}B)\ \text{i.e.}$$

$$R(A\text{-}B) = 2\ \{\ [SD(L_A)^2 + SD(L_B)^2] + [SD(r_A)^2 + SD(r_B)^2]\ /\ 5\}^{1/2}$$

where:

‣ $SD(L_A)$ and $SD(L_B)$ are the Standard Deviations among laboratories for samples A and B.

‣ $SD(r_A)$ and $SD(r_B)$ are the Standard Deviations between replicates, pooled over laboratories and experiments.

‣ Mean A-B is the mean difference between samples A and B, averaged across laboratories.

Resorcinol and benzo*[a]*pyrene have been used to further illustrate the approach to investigating the among laboratory variability. It can be seen that the yields from 2R4F (sample A) and 1R5F (sample B) do not overlap for benzo*[a]*pyrene between any laboratories. However, when the yields from 2R4F (sample A) and 1R5F (sample B) for resorcinol are studied, it can be seen that they do overlap between some laboratories, see Figures A4 and A5.

Another illustration is given below for B*[a]*P and resorcinol that are capable of good and poor differentiation between the two reference cigarettes i.e. expressing data in terms of the mean of (2R4F – 1R5F) and then evaluating the difference between the
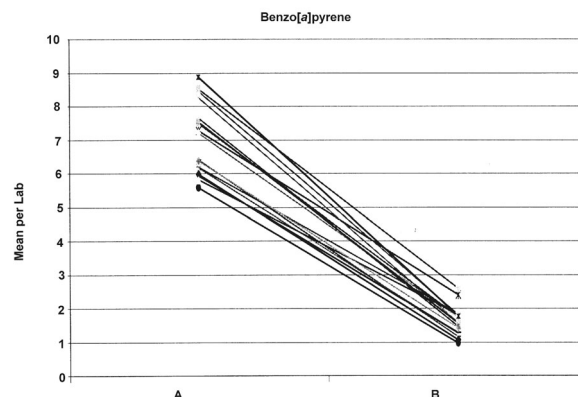
Figure A4. Statistical analysis of benzo[*a*]pyrene yields in 1R5F and 2R4F cigarettes
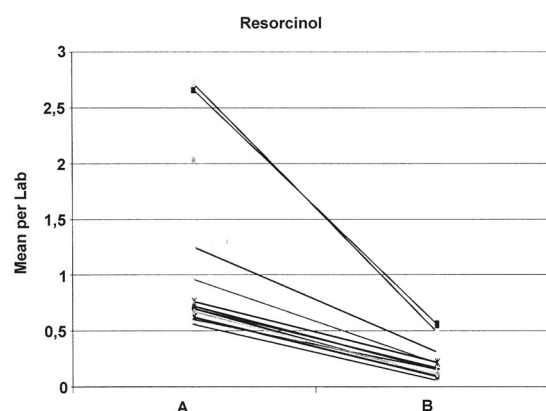
Figure A5. Statistical analysis of resorcinol yields in 1R5F and 2R4F cigarettes

highest and lowest values [*indication* of among laboratory variability (*R*)] as a percentage of this mean. The B*[a]*P value is less than 100% showing that the products are generally differentiated, Figure A6. For resorcinol, products were not well differentiated and give values in excess of 200%, Figure A7.
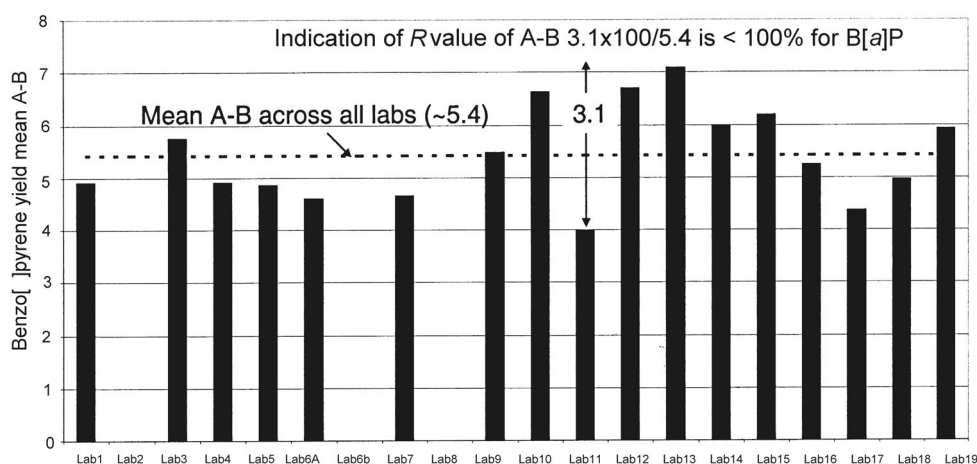
Figure A6. Statistical analysis for benzo[*a*]pyrene yields, differentiation of 1R5F (A) and 2R4F (B) cigarettes; benzo[*a*]pyrene A-B: %CoV = 100 × *R* (A-B)/(Mean A-Mean B)
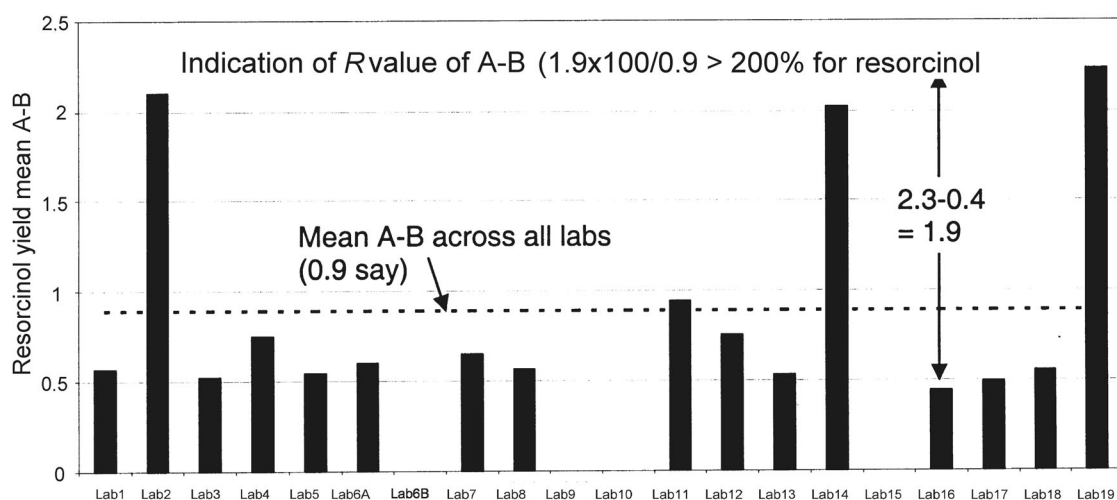
Figure A7. Statistical analysis for resorcinol yields; differentiation of 1R5F (A) and 2R4F (B) cigarettes; resorcinol A-B: %CoV = 100 × R (A-B)/(Mean A-Mean B)

Appendix 12. Differentiation between 1R5F and 2R4F
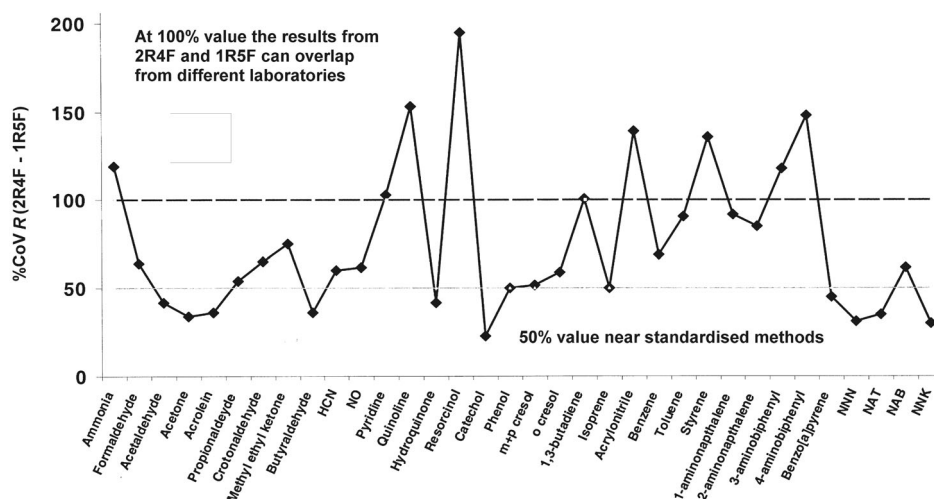
Figure A8. Differentiation between 1R5F and 2R4F cigarettes for different analytes where R (2R4F-1R5F) = the smallest difference between the mean yields for 2R4F and 1R5F (samples A and B) that can be distinguished, with 95% confidence, when each sample has been tested by separate laboratories [% CoV of R (Mean 2R4F – 1R5F) normalised as % coefficient of variation to allow plots of all analytes on one graph], At 100% value the results from 2R4F and 1R5F can overlap from different laboratories

Appendix 13. Effects on variability of the number of cigarettes per peplicate
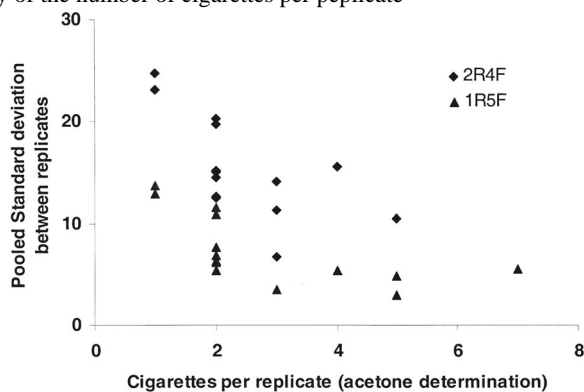
Figure A9. Effects on variability of the number of cigarettes (2R4F - 2R5F) per replicate (acetone determination)

Appendix 14.   Summary of total particulate matter and puff count, 2R4F data

Table A13. Total particulate matter and puff count for 2R4F data obtained in laboratories 1–19 (bases = semi-volatiles (pyridine and quinoline))

Analyte group	Number of labs providing data		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Mean
	Analyte yields	TPM yields																				
Ammonia	12	9	10.8		9.5	10.3			11.0					10.6		10.0		10.8	11.8	10.4		10.6
Aromatic amines	13	11	10.5			10.0	11.2	10.6	11.2		10.4			NP	10.9			10.4	11.5	10.5	10.9	10.7
B[a]P	16	16	10.7		10.0	11.3	11.0	11.1	11.4		10.4	11.6		10.5	11.2	11.0	10.9	10.3	11.3	10.4	10.2	10.8
Carbonyls	16	0																				
HCN	15	8	11.4	9.8		11.7			11.2		9.7			10.4	10.9	9.7		10.1		10.5		10.7
NO	13	8	11.3	9.4	10.3	12.1			11.2					10.4						10.5		10.6
Phenolics	18	17	11.1	10.3	10.4	11.7	12.0	10.6	11.1	11.5	10.4	11.8		10.3	10.9	10.7		10.1	11.4	10.4	10.9	10.9
Volatiles	15	13	10.8	10.5		10.7	10.8	10.6	11.9		10.8	11.4		10.3	11.2			11.1	12.0	10.4	10.7	11.0
Bases	15	12	10.8			10.7		13.4	11.9	12.5		11.4		10.3	10.9			11.1	12.0		10.9	11.4
TSNAs	13	13	11.2		10.6	10.1	11.1	10.0	11.4		10.4	11.4		10.5	10.9			10.3	11.3	10.3	10.9	10.7

Analyte group	Number of labs providing data		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Mean
	Analyte yields	Puff count																				
Ammonia	12	11	8.5		8.5	8.3	8.6	9.2	8.4					8.4		8.9		8.8	9.1	9.1		8.7
Aromatic amines	13	12	8.1		8.7	8.0	8.3	9.2	9.0		8.3			8.7	8.9			8.1	8.8	9.1	8.4	8.6
B[a]P	16	16	8.4		8.5	8.8	8.1	9.2	8.9		8.3	9.2		8.6	9.0	8.5	8.6	8.7	8.5	9.1	8.3	8.7
Carbonyls	16	14	8.6	8.7	9.5	9.0	8.6	9.4	8.5	8.5	8.4			8.5				9.4	10.3	9.2	8.4	8.9
HCN	15	12	8.2	8.8	8.7	9.0	8.3	9.1	8.4		8.3			8.8	9.1			8.3		9.1		8.7
NO	13	12	8.6	11.1	8.4	9.6	8.2	9.3	8.2		8.3			8.2		8.0		8.5		9.1		8.8
Phenolics	18	17	8.7	8.7	8.5	8.8	8.8	8.9	8.8	8.7	8.3	9.5		8.6	9.1	8.9		8.7	8.7	9.1	8.4	8.8
Volatiles	15	14	8.4	8.6		8.1	8.7	8.7	8.6		8.2	8.8		8.5	8.9			8.6	8.4		8.3	8.5
Bases	15	13	8.4			8.2	8.7	8.4	8.5	8.8		8.8		8.5	8.7			8.6	9.2	9.1	8.3	8.6
TSNAs	13	13	8.5		8.9	8.1	8.2	8.8	8.9		8.3	9.2		8.4				8.6	8.8	9.0	8.3	8.6

Appendix 15. Summary of total particulate matter and puff count, 1R5F data

Table A14. **Total particulate matter and puff count for 1R5F data obtained in laboratories 1–19** (bases = semi-volatiles (pyridine and quinoline))

Analyte group	Number of labs providing data		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Mean
	Analyte yields	TPM yields																				
Ammonia	12	9	1.9		1.8									2.6		2.3		2.6	2.0	2.0		2.2
Aromatic amines	13	11	1.9				2.7	2.1	2.5		2.3				2.0			2.3	2.1	2.0	2.4	2.2
B[a]P	16	16	2.4		2.5	2.5	2.6	2.1	2.9		2.4	2.6		2.3	2.1	2.5	2.7	1.8	2.0	2.0	2.4	2.4
Carbonyls	16	0																				
HCN	15	8	2.1			2.3									1.7	3.5		2.0		2.0		2.3
NO	13	8	2.3	2.1	2.1	2.4			2.3		2.4			2.4					2.0	2.0		2.3
Phenolics	18	17	2.2	2.1	2.5	2.4	2.4	1.9	2.6	2.1	2.4	3.2		2.2	2.0	2.1		1.9	2.0	2.0	2.3	2.3
Volatiles	15	13	2.2	2.5		2.5	2.3	2.1	2.8		2.7	2.4		2.3	1.9			2.7	2.1		2.4	2.4
Bases	15	12	2.2			2.4		5.0	2.9	2.5		2.4		2.3	1.8			2.5	2.5	2.0	2.4	2.5
TSNAs	13	13	2.3		2.5	2.3	2.7	2.3	2.6		2.5	2.4		2.4				2.1	2.2	1.8	2.4	2.3

Analyte group	Number of labs providing data		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Mean
	Analyte yields	Puff count																				
Ammonia	12	11	6.9		6.7	6.4	6.9	7.4	6.4					6.8		7.2		7.0	7.2	7.2		6.9
Aromatic amines	13	12	6.4	6.8	6.8	6.1	6.5	6.9	6.5		6.6			6.8	7.1			7.5	7.0	7.3	6.5	6.8
B[a]P	16	16	6.9		6.6	6.9	6.4	7.1	7.0		6.6	7.1		7.0	7.2	7.2	6.6	6.8	7.4	7.1	6.5	6.9
Carbonyls	16	14	6.6	6.9	7.5	6.9	7.0	7.1	6.7	7.0	6.8			6.8	7.2	7.2		7.4	7.8	7.2	6.9	7.0
HCN	15	12	7.2	7.0	7.0	7.1	7.0	7.3	6.6		6.9			7.0	7.4			6.7	7.4	7.4		7.0
NO	13	12	7.1	9.0	6.6	7.6	6.3	6.9	6.1		6.7			6.6		7.0		6.9		7.3		7.0
Phenolics	18	17	6.9	6.8	6.6	6.9	7.1	7.0	6.7	7.1	6.7	7.0		6.9	7.3	6.7		6.7	6.9	7.2	6.9	6.9
Volatiles	15	14	6.7	6.8	6.9	6.1	7.1	7.1	6.7		6.6	7.0		6.8	7.1			6.9	7.4		6.5	6.8
Bases	15	13	6.7			6.4	7.0	6.6	6.7	7.1		7.0		6.8	7.0			6.9	7.1	7.2	6.5	6.8
TSNAs	13	13	6.9			6.2	6.5	7.0	6.9		6.5	7.0		7.0				6.9	7.1	7.1	6.5	6.8

Appendix 16. Overview of ammonia methodology

Table A15. Overview of ammonia methodology in several laboratories (AA = auto analyser; C = conductivity detector; CFP = Cambridge filter pad; EP = electrostatic precipitation; IC = ion chromatography; Imp = impinger; SF = separatory funnel; P = photometer)

Methodology	Laboratory											
	1	4	5	6	7	11	12	14	16	17	18	3
No. cigs per replicate	3	10	5	5 or 10	5	20	20	10	5	10 or 20 (LT)	4	20
Type of smoking												
Linear or rotary	linear	rotary	linear	linear	linear	rotary	rotary	rotary	rotary	rotary	linear	rotary
Make	Cerulean SM450	Borgwaldt RM20	Borgwaldt KC20X	Filtrona SM350	KC10	Borgwaldt RM200	Borgwaldt RM20/ RM20H	Borgwaldt	Borgwaldt RM20CSR	Borgwaldt RM20CSR	Internal	Borgwaldt RM20
Trapping system												
Filter and diameter	CFP 44 mm	CFP 92 mm	no	no	CFP 44 mm	CFP 92 mm	CFP 92 mm	CFP 92 mm	CFP 92 mm	CFP 44 mm	CFP 44 mm	EP
Trap type	1 Imp	2 Imp	2 Imp	1 SF	2 Imp	1 Imp	3 Imp	1 Imp	2 Imp	solid sorbent tube	1 Imp	1 Imp
Filtration step	yes	yes	no	yes	no	yes	yes	no	no	yes	yes	no
Derivatisation												
Reagent	no	no	no	no	no	no	no	no	no	no	no	ninhydrin
Analytical equipment												
Autosampler	yes	no	yes	yes	yes	no		no	yes		no	
Cooling? and temperature	10 ˚C	RT		6 ˚C					4 ˚C			
Description	IC	IC	IC	IC	IC	IC	IC	IC	IC	IC	IC	AA
Conditions	isocratic	isocratic	gradient	isocratic	isocratic	isocratic	isocratic	isocratic	gradient	isocratic	gradient	
Detection	C	C	C	C	C	C	C	C	C	C	C	P
Calibration curve	linear	quadratic	quadratic	quadratic	linear	linear		linear	linear	quadratic	linear	linear

Appendix 17. Ammonia data
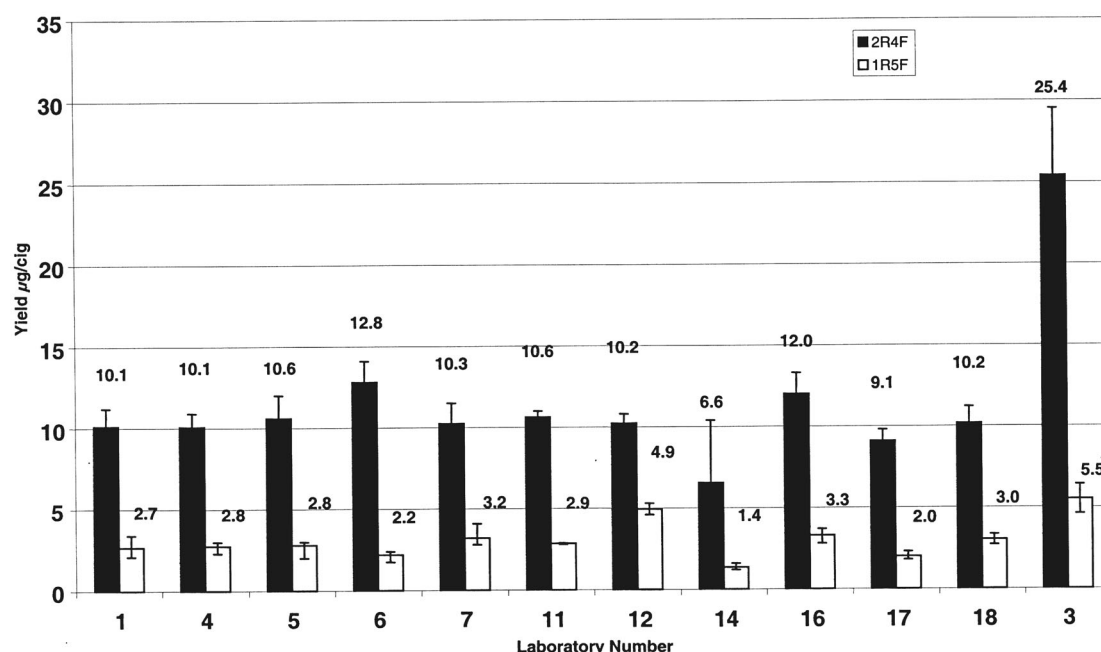
Figure A10. Ammonia yields (µg/cig) obtained in different laboratories for 2R4F and 1R5F cigarettes. Error bars indicate the maximum and minimum yields given by a laboratory

Table A16. Overview of aromatic amines methodology in several laboratories (CFP = Cambridge filter pad; DCM = dichloromethane; GC-MS = gas chromatography-mass spectrometry; HFBA = heptafluorobutyric acid anhydride; PFPA = pentafluoropropionic acid anhydride; RT = room temperature; TMA = trimethylamine;)

Methodology	Laboratory												
	1	3	4	5	6	7	9	12	13	16	17	18	19
No. cigs per replicate	5 or 10	10	10	20	5	5	5	10	10	10	5	20	10
Type of smoking													
Linear or rotary		rotary	rotary	rotary	linear	linear	rotary	rotary	linear	rotary	linear	linear	linear
Make		Borgwaldt RM20	Borgwaldt RM200	Borgwaldt RM200	Cerulean SM450 & SM 350			Borgwaldt RM20/ RM20H	Cerulean SM450	Borgwaldt	Cerulean SM400	Internal	
Trapping system													
Filter pad or glass trap	CFP 44 mm	5 im pingers	CFP 92 mm	CFP 92 mm	CFP 44 mm	CFP 44 mm	CFP 44 mm	3 impingers	CFP 44 mm	CFP 92 mm	CFP 44 mm	CFP 44 mm	CFP 44 mm
Stage at which IS is added	before DCM partition	before DCM partition	before DCM partition	before DCM partition	after smoking, before extraction	after smoking, before extraction	after extraction from CFP	before DCM partition	before DCM partition	after DCM partitiomnm	after extraction from CFP	after extraction from CFP	
Derivatisation													
Reagent	TMA + PFPA	PFPA	TMA + PFPA	HFBA	HFBA	TMA + PFPA	TMA + PFPA	PFPA	TMA + PFPA	TMA + PFPA	TMA + PFPA	TMA + PFPA	
Temperature	RT	RT	RT	80 °C	80 °C	RT	RT	RT	RT	RT	RT	RT	RT
Time period	overnight	>1 hour	>60 min	30 min	30 min	1 hour	15 min	30 min	3 h overnight	overnight	30 min	60 min	
Clean-up													
Column	yes	yes	yes	yes	yes	yes	no	yes	yes	yes	no	yes	
Analytical													
Description	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS-MS	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS

Appendix 19. Aromatic amines – yield patterns of the 4 individual amines 1-amino naphthalene (1-NA), 2-amino naphthalene (2-NA), 3-amino biphenyl (3-AB), 4-amino biphenyl (4-AB)

Figure A11. Aromatic amines yield patterns of the 4 individual amines 1-NA = 1-amino naphthalene, 2-NA = 2-amino naphthalene, 3-AB = 3-amino biphenyl, 4-AB = 4-amino biphenyl for 2R4F and 1R5F cigarettes. Error bars give the maximum and minimum yields given by a laboratory expressed as an average of all replicates. Major methodological differences are highlighted (Impinger = use of impinger trapping system; other laboratories trapped on Cambridge filter pads; HFBA = heptafluorobutyric acid anhydride; other methods used pentafluoropropionic acid anhydride).

Appendix 20. Aromatic amines – yield ratios of the 4 individual amines 1-amino naphthalene (1-NA), 2-amino naphthalene (2-NA), 3-amino biphenyl (3-AB), 4-amino biphenyl (4-AB)
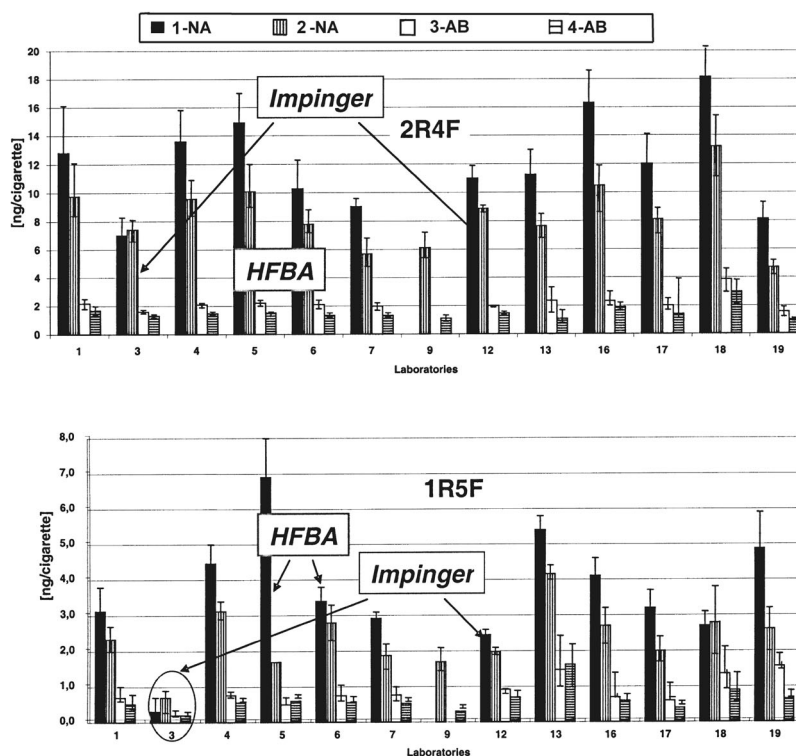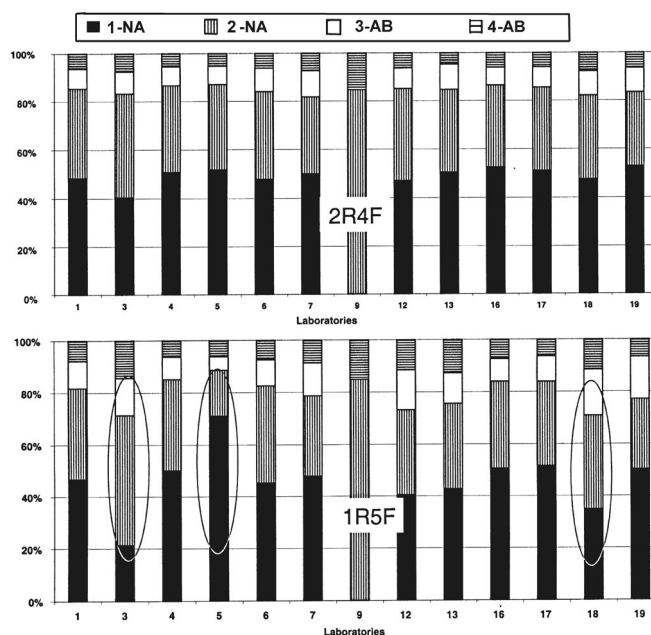

Figure A12. Aromatic amines yield ratios of the 4 individual amines 1-NA = 1-amino naphthalene, 2-NA = 2-amino naphthalene, 3-AB = 3-amino biphenyl, 4-AB = 4-amino biphenyl for 2R4F and 1R5F cigarettes. Yield ratios are the yields of the four aromatic amines summed together with individual amine yields then expressed as percentages of the total yield. The different yield ratio patterns observed are unrelated to specific methods. Note that laboratory 9 measured only 2-amino naphthalene and 4-aminobiphenyl.

Appendix 21. Overview of benzo[*a*]pyrene methodology

Table A17. Overview of benzo[a]pyrene methodology CFP = Cambridge filter pad; F = fluorescence; GC-MS = gas chromatography-mass spectrometry; HPLC = high performance liquid chromatography; SIM = single ion monitoring)

| Methodology	Laboratory								
	1	5	7	9	10	11	12	13	14
No. cigs per replicate	5	20	5 to 10	10 or 20	10	10	10	10	5
Type of smoking machine									
Linear or rotary	linear	rotary	linear	rotary	rotary	rotary	rotary	linear	rotary
Make	Cerulean SM450	Borgwaldt RM200	n/p	Borgwaldt	Borgwaldt RM20 CSR	Borgwaldt RM20 CSR	Borgwaldt RM20/RM20H	Cerulean SM450	Borgwaldt
Trapping system									
Filter and diameter	CFP 44 mm	CFP 92 mm	CFP 44 mm	CFP 44 mm	CFP 92 mm	CFP 44 mm	CFP 44 mm	CFP 44 mm	CFP 92 mm
Clean-up									
Column	yes	yes	yes	yes	yes	yes	yes	yes	yes
Analytical equipment									
Description	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS
Detection	SIM	SIM	SIM	SIM	SIM		MS	SIM	SIM

Methodology	Laboratory							
	17	18	19	3	4	6	15	16
No. cigs per replicate	5	20	20	20	5	5	20	5
Type of smoking machine								
Linear or rotary	linear	linear	linear	rotary	linear	linear	rotary	linear
Make	Cerulean SM400	Internal	n/p	Borgwaldt RM20	Internal	Cerulean SM450/ SM350	Borgwaldt RM20	Filtrona
Trapping system								
Filter and diameter	CFP 44 mm	CFP 44 mm	CFP 44 mm	CFP 92 mm	CFP 44 mm	CFP 44 mm	CFP 92 mm	CFP 44 mm
Clean-up								
Column	yes	yes	yes	yes	yes	x	yes	yes
Analytical equipment								
Description	GC-MS	GC-MS	GC-MS	HPLC	HPLC	HPLC	HPLC	HPLC
Detection	SIM	SIM	MS	F	F	F	F	F

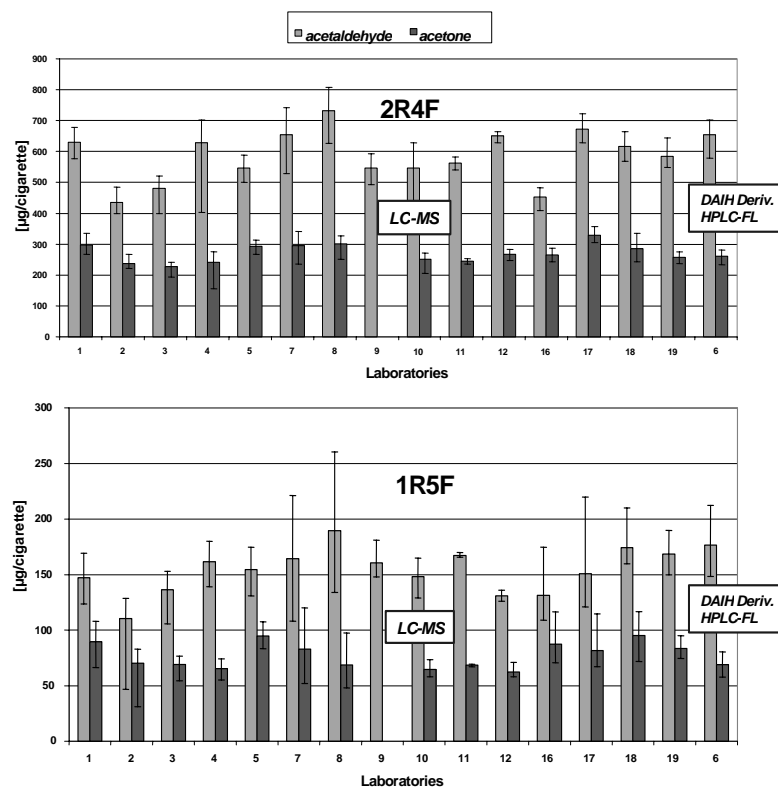Appendix 22. Benzo[*a*]pyrene yields
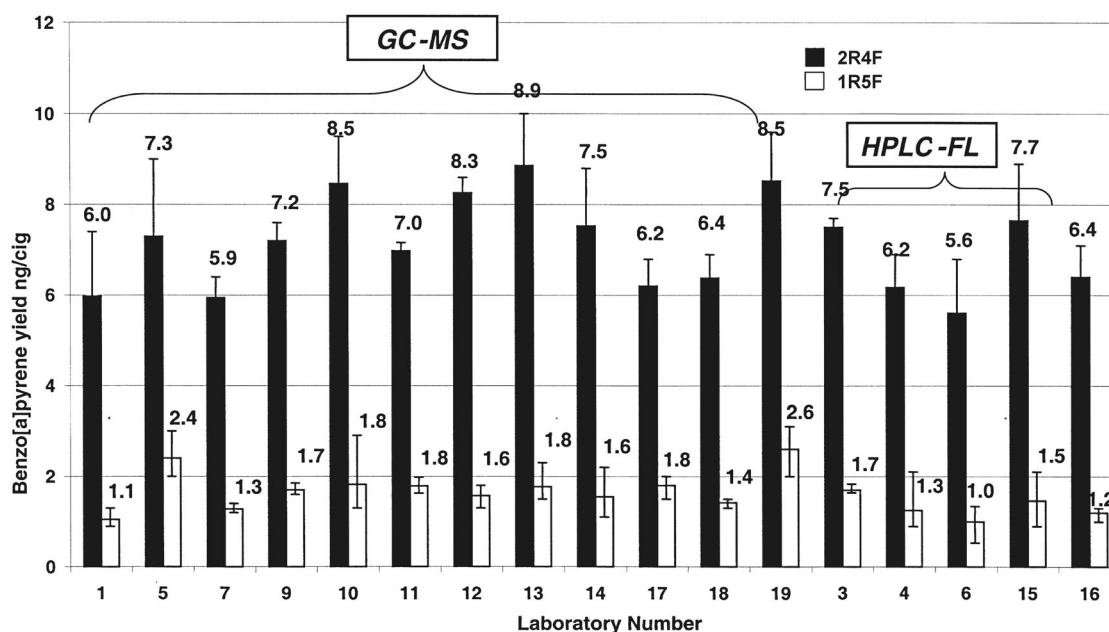
Figure A13. Benzo[*a*]pyrene yields (ng/cig) obtained in different laboratories for 2R4F and 1R5F cigarettes. Error bars are maximum and minimum values given by one laboratory (GC-MS = gas chromatography – mass spectrometry; HPLC-FL = high performance liquid chromatography - fluorescence detection).

Table A17. Overview of carbonyl methodology (AA = autoanalyser [formaldehyde only]; C = colorimeter; DAD = diode array detector; DNPH = 2,4-dinitrophenylhydrazine; DPAIH = 2-diphenylacetyl-1,3-indanedione-1-hydrazone; F = fluorescence; MWD = multi-wavelength detector; PDA = photo diode arry detector; RT = room temperature; UV = ultra-violet)

Methodology	Laboratory																
	1	2	3	4	5	7	8	9	10	11	12	16	17	18	19	6	14
No. cigs per replicate	3	2	5	2	2	1	1	10	4	2	3 to 5	2	2	2	1	3 to 7	3
Type of smoking machine																	
Linear or rotary	linear	linear	rotary	linear	linear	linear	linear	rotary	rotary	linear	linear	linear	linear	linear	linear	linear	linear
Make	Cerulean ASM450	Borgwaldt KC20X	Borgwaldt RM200		Borgwaldt KC20X	Borgwaldt KC10	Filtrona 20 port	Borgwaldt	Borgwaldt RM20 CSR	Cerulean SM400/45 0	Borgwaldt 4 channel	Filtrona	Cerulean SM350	Internal	X	Hawktech MB 1000	Cerulean ASM500
Trapping system																	
Filter and diameter	no	CFP 44 mm	no	no	no	CFP 44 mm	no	no	no	no	no	no	no	CFP 44 mm	CFP 44 mm	no	CFP
Trap type + no. of traps	1 Imp	2 Imp	2 Imp	1 Imp	2 Imp	2 Imp	2 Imp	2 wash bottles	2 Imp	1 Imp	2 Imp	1 Imp	1 Imp	filter trap + two DNPH-treated FPs	filter trap + filter trap + two DNPH-treated FPs	1 Imp	activated silica gel
Derivatisation																	
Reagent	DNPH	DNPH	DNPH	DNPH	DNPH	DNPH	DNPH	DNPH	DNPH	DNPH	DNPH	DNPH	DNPH	DNPH	DNPH	DPAIH	
Temperature	RT	RT	RT	RT	RT	RT	RT	RT	RT	ice water			RT			RT	RT
Time to reaction end (min)	5 to 20		30	<60	120	10	directly after smoking	<5	10	30			10	directly after smoking	directly after smoking	directly after smoking	30
Column cleanup	no	yes	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
Analytical equipment																	
Description	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC	LC-MS-MS								
Detection	UV	UV	DAD	DAD	UV	UV	PDA	DAD	MS-MS								

Appendix 24. Yields of the major carbonyls acetaldehyde and acetone

Figure A14. Yields of major carbonyls acetaldehyde and acetone obtained in different laboratories for 2R4F and 1R5F cigarettes Error bars give the maximum and minimum values for each laboratory (2-diphenylacetyl-1,3-indandione-1-hydrazone (DPAIH) derivative HPLC-FL = diphenylacetylhydrazone derivative measured by high performance liquid chromatography with fluorescence detection).

Appendix 25. Yield ratios of the major carbonyls acetaldehyde and acetone
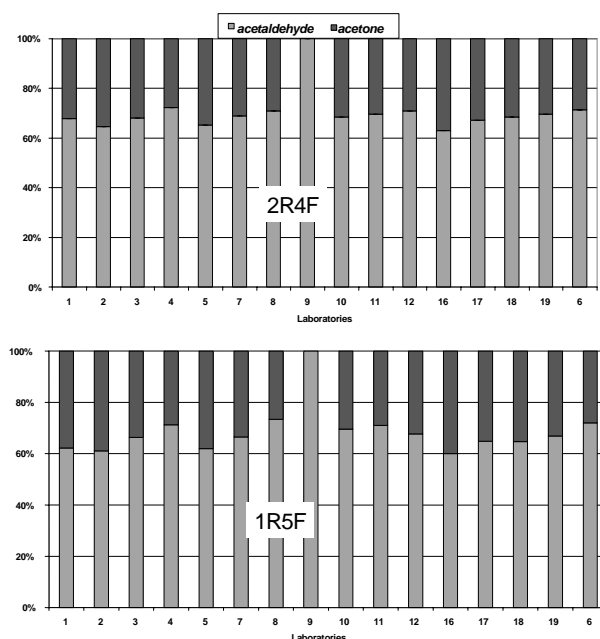
Figure A15. Yield ratios of major carbonyls acetaldehyde and acetone obtained in different laboratories for 2R4F and 1R5F cigarettes. The yields of acetaldehyde and acetone were summed together and expressed as individual percentages of the total yield. Note that Laboratory 9 did not measure acetone.

Appendix 26. Yields of the minor carbonyls formaldehyde, acrolein, methyl ethyl ketone (MEK), propionaldehyde, butyraldehyde and crotonaldehyde

Figure A16. Yields of minor carbonyls formaldehyde, acrolein, methyl ethyl ketone (MEK), propionaldehyde, butyraldehyde and crotonaldehyde obtained in different laboratories for 2R4F and 1R5F cigarettes (LC-MS = liquid chromatography – mass spectrometry; 2-diphenylacetyl-1,3-indandione-1-hydrazone (DPAIH) derivative HPLC-FL = diphenylacetylhydrazone derivative measured by high performance liquid chromatography with fluorescence detection).

Appendix 27. Yield ratios of the minor carbonyls formaldehyde, acrolein, methyl ethyl ketone (MEK), propionaldehyde, butyraldehyde and crotonaldehyde
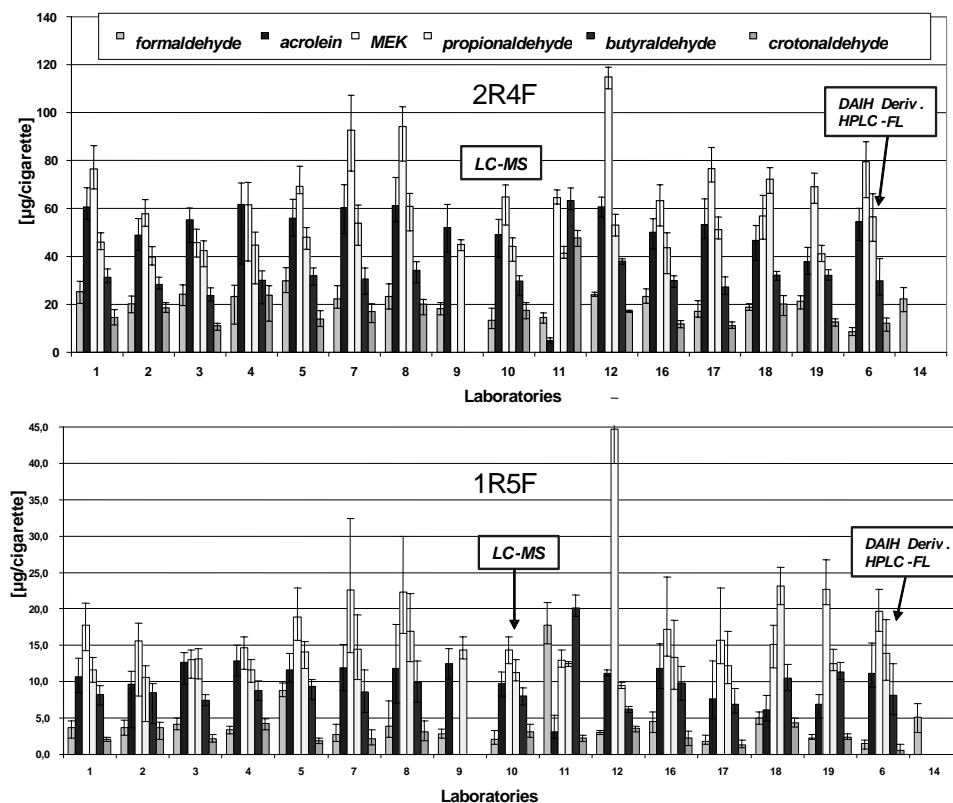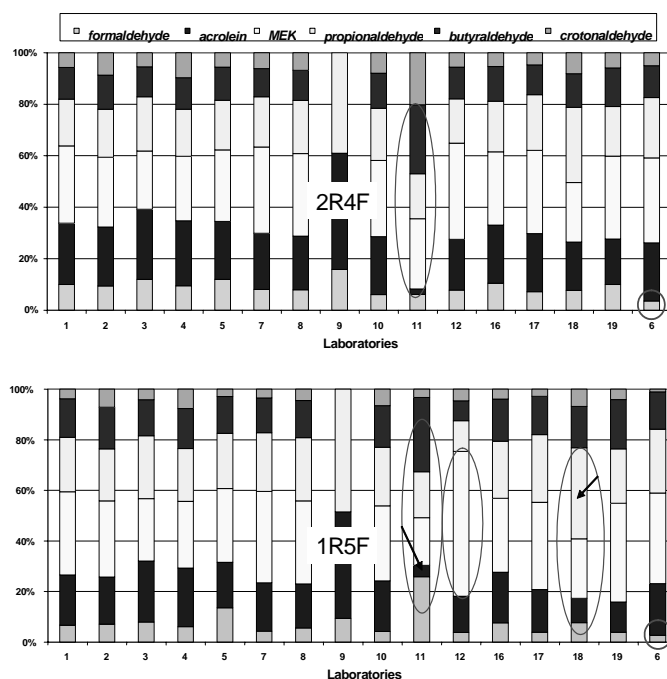

Figure A17. Yield ratios of minor carbonyls formaldehyde, acrolein, methyl ethyl ketone (MEK), propionaldehyde, butyraldehyde and crotonaldehyde.
The yields of the six minor carbonyls were summed together and expressed as individual percentages of the total yield as yield ratios. The major differences are highlighted.

192

Appendix 28. Overview of hydrogen cyanide methodology

Table A18. Hydrogen cyanide methodolgy (LC-MS-MS = liquid chromatography-mass spectrometry-mass spectrometry; NDA = 2,3-naphthalene dicarboxaldehyde; NPD = nitrogen phosphorous detector)

Methodology	Laboratory							
	1	2	4	5	6	7	13	14
No. cigs per replicate	3 to 5	5	5	5	5	3	5 to 20	3
Type of smoking machine								
Linear or rotary	linear	linear	linear	linear	linear	linear	rotary	linear
Make	Cerulean ASM450	Borgwaldt KC20X	Internal	Borgwaldt KC20X	Modified Phipps & Byrd	Borgwaldt KC10	Borgwaldt RM20	Cerulean ASM500
Trapping system								
Filter and diameter	CFP 44 mm	CFP 44 mm	CFP 44 mm	no	CFP 44 mm	CFP 44 mm	CFP 92 mm	CFP trapping unit with activated silicia gel
Trap type + no. of traps	1 Imp	1 Imp	1 Imp	2 Imp	1 Ascarite trap	1 Imp	1 Imp	
Derivatisation								
Measured complex based on		pyrazolone	pyrazolone	pyrazolone	barbituric acid	barbituric acid	pyrazolone	pyrazolone
Analytical equipment								
Description	UV/VIS spectrophotometer	continuous flow analyser	continuous flow analyser	continuous flow analyser	continuous flow analyser	continuous flow analyser	continuous flow analyser	continuous flow analyser
Detection	Colorimeter	Colorimeter	Colorimeter	Colorimeter	Colorimeter	Colorimeter	Colorimeter	Colorimeter
Wavelenght	518	540	630	540	570	575	540	540

Methodology	Laboratory						
	16	18	10	11	12	3	9
No. cigs per replicate	5	3-5	10	20	6	20	10
Type of smoking machine							
Linear or rotary	linear	linear	rotary	rotary	linear	rotary	rotary
Make	Filtrona	Internal	Borgwaldt RM20CSR	Borgwaldt RM200	Borgwaldt 4 channel	Borgwaldt RM20	Borgwaldt
Trapping system							
Filter and diameter	CFP 44 mm	CFP 44 mm	X	CFP 92 mm	X	no	X
Trap type + no. of traps	1 Imp	1 Imp	2 Imp	1 Imp	1 glass tube filled with NaOH on support	glass syringe, individual puffs being sampled	2 Imp
Derivatisation							
Measured complex based on	pyrazolone	barbituric acid	NDA & taurine	no	no	no	addition of phosphoric acid
Analytical equipment							
Description	continuous flow analyser	UV/VIS spectrophotometer	LC-MS-MS	IC	ion sensitive electrode	GC	headspace GC
Detection	Colorimeter	Colorimeter	MS-MS	Conductivity	ion sensitive electrode	FID	NPD
Wavelenght	540	600					

Appendix 29. Hydrogen cyanide yields
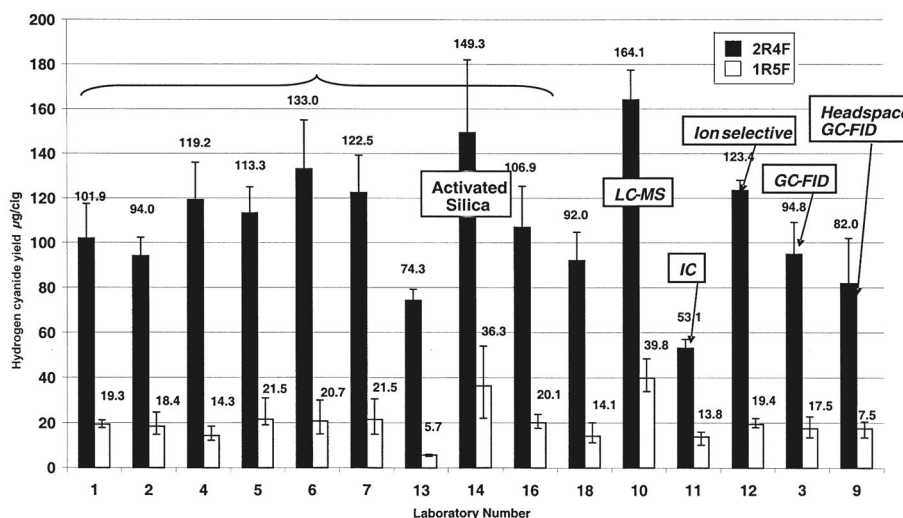
Figure A18. Hydrogen cyanide yields (µg/cig) obtained in different laboratories for 2R4F and 1R5F cigarettes. Error bars give the maximum and minimum yields given by a laboratory expressed as an average. Chloramine T, reaction with pyrazolone or barbituric acid. Detection and quantification by continuous flow analysis or photometer.

Appendix 30. Overview of nitric oxide methodology

Table A19. Nitric oxide methodology

Methodology	Laboratory						
	1	2	3	4	5	6	7
No. cigs per replicate	20	1	20	1	10	10	1
Type of smoking machine							
Linear or rotary	rotary	single port	rotary	single port	rotary	linear	linear
Make	Borgwaldt RM20	Borgwaldt	Borgwaldt RM20	Borgwaldt R26	Borgwaldt RM20	Internal	Borgwald KC5
Trapping system							
Filter and diameter	CFP 92 mm	CFP 44 mm	CFP 92 mm	CFP 44 mm		CFP 44 mm	no
Trap type + no. of traps	puff by puff dilute each puff	puff by puff no dilution	puff by puff dilute each puff	puff by puff dilute each puff	puff by puff no dilution	gas collection bag no dilution	puff by puf dilute each puff
Analytical equipment							
Description	NO analyser (NO)	NO analyser (NO)	NO analyser (NO)	NO analyser (NO)	NO analyser (NO)	NO analyser (NOx)	NO analyser (NO)
Detection system and conditions	chemi-luminescence on full + partial puffs	chemi-luminescence on full puffs only	chemi-luminescence on full + partial puffs	chemi-luminescence on full + partial puffs	chemi-luminescence on full + partial puffs	chemi-luminescence on full + partial puffs	chemi-luminescence on full + partial puffs

Methodology	Laboratory					
	9	11	12	14	16	18
No. cigs per replicate	5	2 × 10	7	10	1	10
Type of smoking machine						
Linear or rotary	rotary	rotary	linear	rotary	singel port	linear
Make		Borgwaldt RM20CSR	Borgwaldt 4 channel	Borgwaldt	Borgwaldt	Internal
Trapping system						
Filter and diameter	CFP 44 mm	CFP 92 mm		CFP 92 mm	no	CFP 44 mm
Trap type + no. of traps	puff by puff dilute each puff	puff by puff no dilution	puff by puff	puff by puff no dilution	puff by puff dilute each puff	gas collection bag no dilution
Analytical equipment						
Description	NO analyser (NO)	NO analyser (NOx)	NO analyser	NO analyser (NO)	NOx analyser (NO)	NOx analyser (NO)
Detection system and conditions	chemi-luminescence on full + partial puffs	chemi-luminescence on full puffs only	chemi-luminescence on full + partial puffs	chemi-luminescence on full puffs only	chemi-luminescence on full + partial puffs	chemi-luminescence on full + partial puffs

Appendix 31. Nitric oxide yields
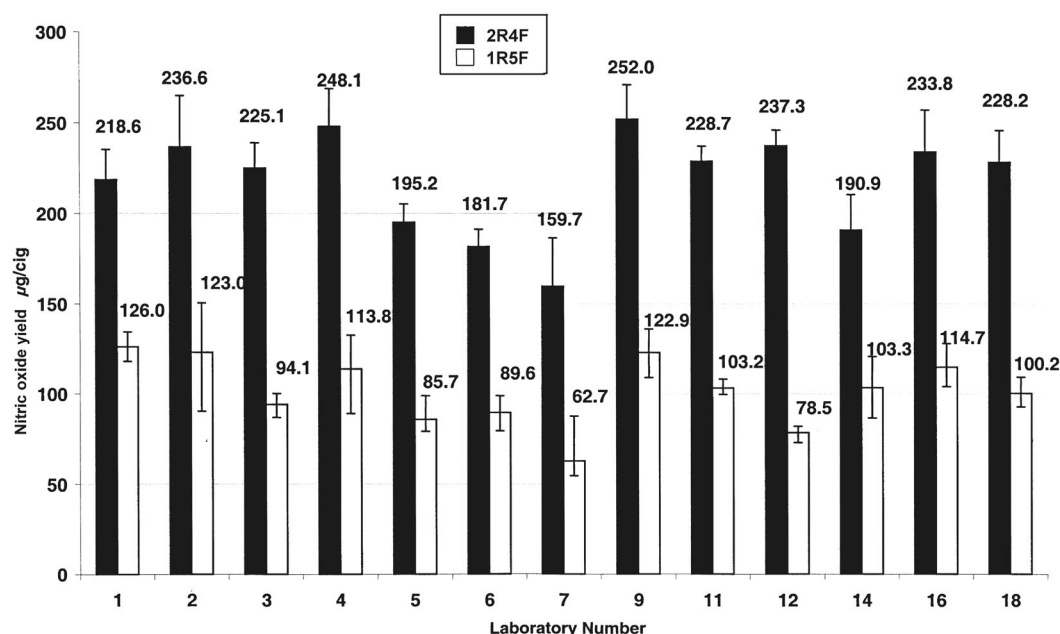
Figure A19. Nitric oxide yields (µg/cig) obtained in different laboratories for 2R4F and 1R5F cigarettes. Error bars give the maximum and minimum yields given by a laboratory expressed as an average of all replicates.

Appendix 32. Overview of phenolics methodology

Table A19. Phenolics methodology (BSTFA = bis(trimethylsilyl)trifluoroacetamide; TMCS = trimethylchlorosilane; PDA = photo diode array; HPLC = high performance liquid chromatography; GC-MS = gas chromatography-mass spectrometry; SIM = single ion monitoring)

Methodology	Laboratory								
	1	2	3	4	6	7	10	11	12
No. cigs per replicate	5-10	20	20	5	5	5	20	5	3 to 5
Type of smoking machine									
Linear or rotary	linear	linear	rotary	linear	linear	linear	rotary	rotary	linear
Make	Cerulean ASM500	Borgwaldt KC20X	Borgwaldt RM20	Internal	Cerulean SM450/ Filtrona SM350		Borgwaldt RM20	Borgwaldt RM20 CSR	Borgwaldt 4 channel
Trapping system									
Filter and diameter	CFP 44 mm	CFP 44 mm	CFP 92 mm	CFP 44 mm	CFP 44 mm	CFP 44 mm	CFP 92 mm	CFP 44 mm	CFP 44 mm
Extraction									
Extraction solvent	1% acetic acid	1% acetic acid	1% acetic acid	1% acetic acid	1% acetic acid	1% acetic acid + methanol	1% acetic acid	1% acetic acid	1% acetic acid
Filtered?	yes	yes	no	yes	yes	no	no	no	yes
Derivatisation									
Reagent	no	no	no	no	no	no	no	no	no
Conditions	no	no	no	no	no	no	no	no	no
Analytical equipment									
Description	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC
Conditions	gradient	isocratic	gradient	gradient	gradient	gradient	gradient	gradient	gradient
Detection	fluorescence	fluorescence	fluorescence	fluorescence	fluorescence	fluorescence	fluorescence	PDA	fluorescence

Methodology	Laboratory							
	13	14	16	17	18	19	5	9
No. cigs per replicate	10	5	5	5	4	5	5	10
Type of smoking machine								
Linear or rotary	linear	linear	linear	linear	linear	linear	rotary	rotary
Make	Cerulean SM450	Cerulean ASM500	Filtrona	Cerulean SM400	Internal		Borgwaldt RM20	Borgwaldt RM20 CSR
Trapping system								
Filter and diameter	CFP 44 mm	CFP 44 mm	CFP 44 mm	CFP 44 mm	CFP 44 mm	CFP 44 mm	CFP 44 mm	CFP 44 mm
Extraction								
Extraction solvent	1% acetic acid	propan-1-ol	1% acetic acid	1% acetic acid	1% acetic acid	1% acetic acid	tert-butyl methyl ether	acetone/ chloroform
Filtered?	yes	no	no	yes	yes	no	no	no
Derivatisation								
Reagent	no	no	no	no	no	no	BSTFA	BSTFA + TMCS (1%)
Conditions	no	no	no	no	no	no	76 ˚C for 30 min	70 ˚C for 15 min
Analytical equipment								
Description	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC	GC-MS	GC-MS
Conditions	gradient	gradient	gradient	gradient	gradient	gradient	X	X
Detection	fluorescence	fluorescence	fluorescence	fluorescence	fluorescence	fluorescence	SIM	SIM

Appendix 33. Yields of phenolics
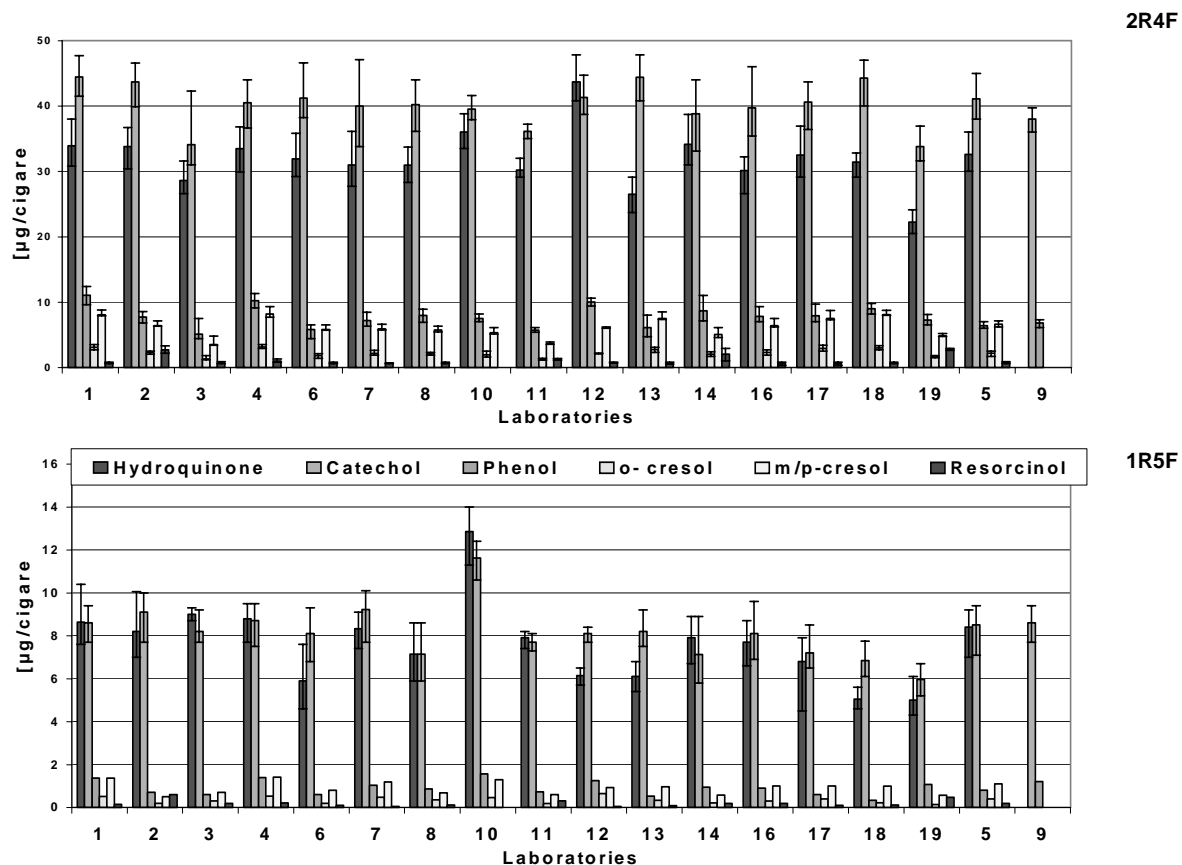
2R4F

1R5F

Figure A20. Phenolics yields (hydroquinone, catechol, phenol, *o*-cresol, *m/p*-cresol, resorcinol) obtained in different laboratories for 2R4F and 1R5F cigarettes. Laboratories 5 and 9 carried out BSTFA derivatisation. Error bars give the maximum and minimum yields given by a laboratory expressed as an average of all replicates

Appendix 34. Ratio patterns of phenolics
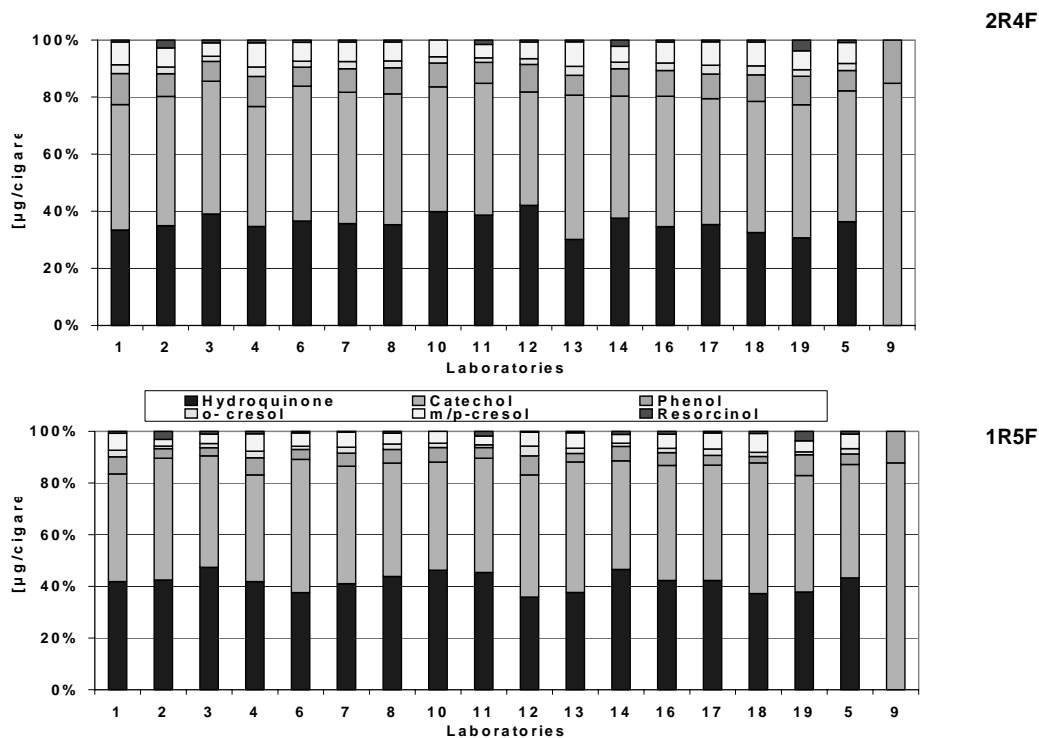
2R4F

1R5F

Figure A21. Ratio patterns of phenolics (hydroquinone, catechol, phenol, *o*-cresol, *m/p*-cresol, resorcinol) obtained in different laboratories for 2R4F and 1R5F cigarettes. The yields of the six phenolics were summed together and expressed as individual percentages of the total yield as yield ratios.
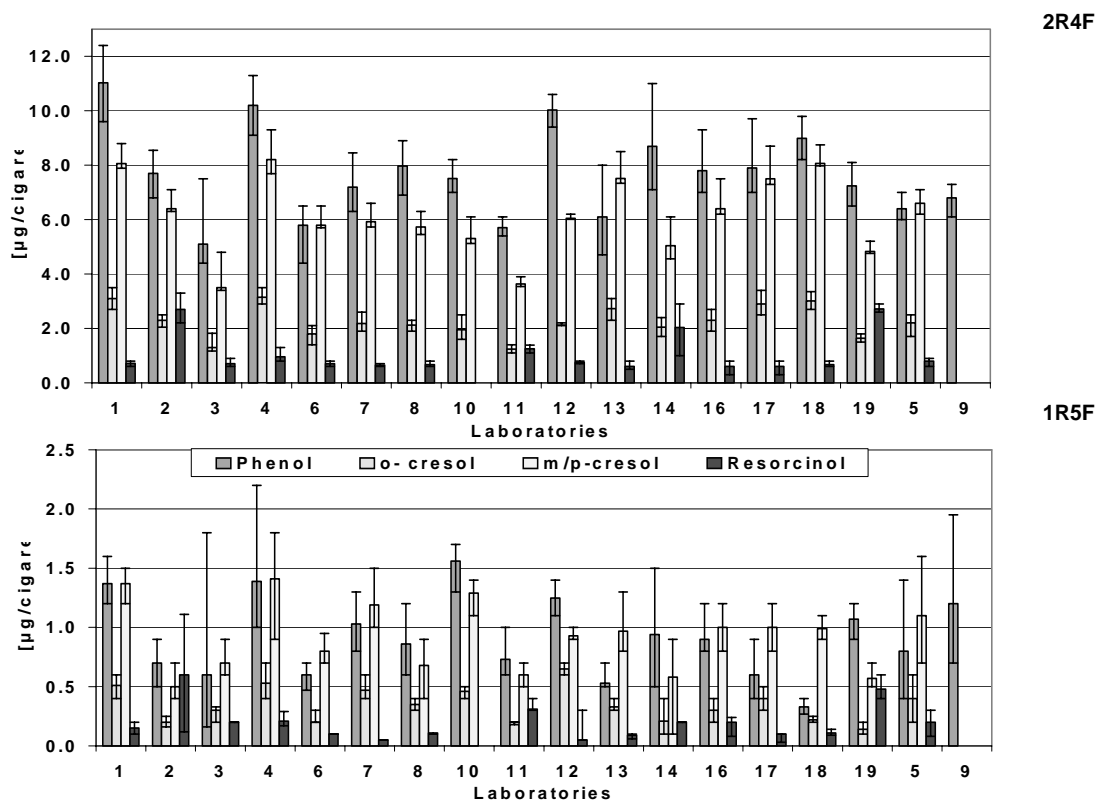
196

Appendix 35. Minor phenolic yields

2R4F

1R5F

Figure A22. Minor phenolic yields (phenol, *o*-cresol, *m/p*-cresol, resorcinol) obtained in different laboratories for 2R4F and 1R5F cigarettes. Error bars indicate the maximum and minimum yields given by a laboratory expressed as an average of all replicates.

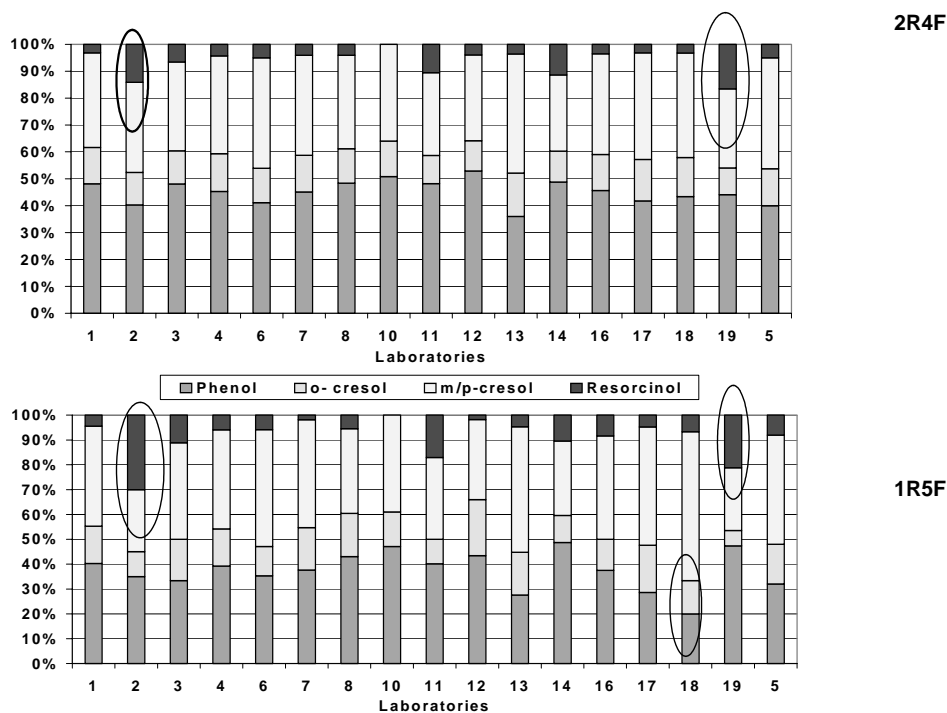Appendix 36 – Ratio patterns of minor phenolics

2R4F

1R5F

Figure A23. Ratio patterns of minor phenolics (phenol, *o*-cresol, *m/p*-cresol, resorcinol) for 2R4F and 1R5F cigarettes. The yields of the four minor phenolics were summed together and expressed as individual percentages of the total yield as yield ratios. Some differences in patterns between 2R4F and 1R5F, see laboratories 2, 18 and 19.

Appendix 37. Overview of methodology for selected volatiles

Table A20. Methodology for selected volatiles (EI = electron ionization; FID = flame ionization detection; SIM = single ion monitoring)

Methodology	Laboratory								
	1	11	1	2	4	5	6A	6B	7
No. cigs per replicate	20	20	5-10	10	10	5	20		5
Type of smoking machine									
Linear or rotary	rotary	rotary	rotary	linear	rotary	rotary	rotary	linear	linear
Make	Borgwaldt RM20	Borgwaldt RM200	Borgwaldt RM20	Borgwaldt KC20X	Borgwaldt RM20	Borgwaldt RM20	Borgwaldt RM20CSR	Borgwaldt RM20 CSR	
Trapping system									
Filter and diameter	X	CFP 92 mm	CFP 92 mm	CFP 92 mm	CFP 92 mm	CFP 44 mm	CFP 92 mm	CFP 92 mm	CFP 44 mm
Trapping	glass syringe individual puffs RT	1 Imp	1 Imp −70 ˚C	2 Imp −70 ˚C	2 Imp −70 ˚C	1 Tedlar bag RT	4 Imp	Tedlar bag RT	1 Imp −70 ˚C
Trapping solvent	no	methanol	methanol	methanol	methanol	no	methanol	methanol	methanol
Extraction									
Filtration step	no	no	no	no	no	no	no	no	no
Clean-up									
Column clean-up step	no	no	no	yes	no	no	no	no	no
Analytical equipment									
Description	GC	GC	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS		GC-MS
Detection system	FID	FID	SIM	MS	SIM	SIM	SIM		MS-EI

Methodology	Laboratory							
	9	10	12	13	16	17	18	19
No. cigs per replicate	10	10	10	10	10	10 or 20	5	10
Type of smoking machine								
Linear or rotary	rotary	rotary	rotary	rotary	rotary	rotary	linear	linear
Make	Borgwaldt 20-port	Borgwaldt RM20CSR	Borgwaldt RM20/RM20H	Borgwaldt RM20	Borgwaldt RM20CS	Borgwaldt RM20CSR		Borgwaldt KC 5 port
Trapping system								
Filter and diameter	CFP 44 mm	CFP 92 mm	CFP 44 mm	CFP 92 mm	CFP 92 mm	CFP 92 mm	CFP 44 mm	CFP 44 mm
Traping	3 Imp −78 ˚C	2 Imp 0 ˚C; −70 ˚C	3 Imp	2 Imp −70 ˚C	1 Imp −70 ˚C	1 Imp −70 ˚C	1 Imp	1 Imp −70 ˚C
Trapping solvent	methanol	methanol	methanol	methanol	methanol	ethanol	methanol	methanol
Extraction								
Filtration step	no	no	yes	no	no	no	no	no
Clean-up								
Column clean-up step	no	no	no	no	no	no	no	no
Analytical equipment								
Description	GC	GC-MS	GC-MS	GC-MS	GC-MS	GC-FID	GC-MS	GC-MS
Detection system	MS	SIM	MS	SIM	MS ion trap positive EI	FID	SIM	MS

Appendix 38. Overview of selected volatiles yields (1,3-butadiene, isoprene, acrylonitrile, benzene, toluene)
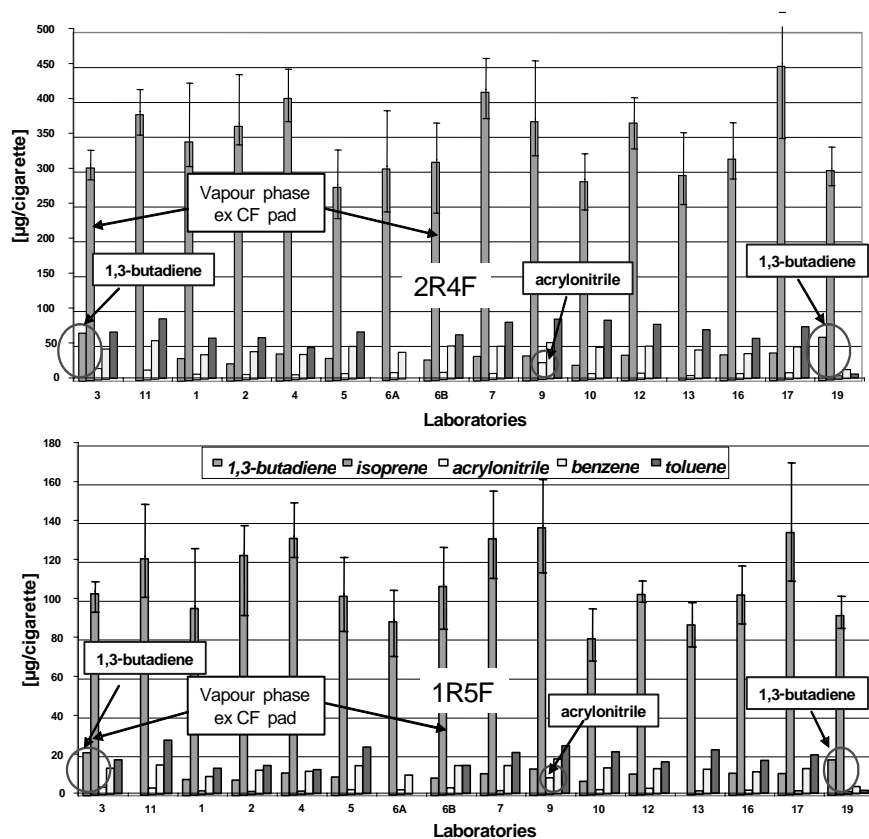
Figure A24. Selected volatiles yields (µg/cig) (1,3-butadiene, isoprene, acrylonitrile, benzene, toluene) for 2R4F and 1R5F cigarettes. Error bars give the maximum and minimum yields given by a laboratory expressed as an average of all replicates.

Appendix 39. Ratio of patterns of selected volatiles (1,3-butadiene, isoprene, acrylonitrile, benzene, toluene)
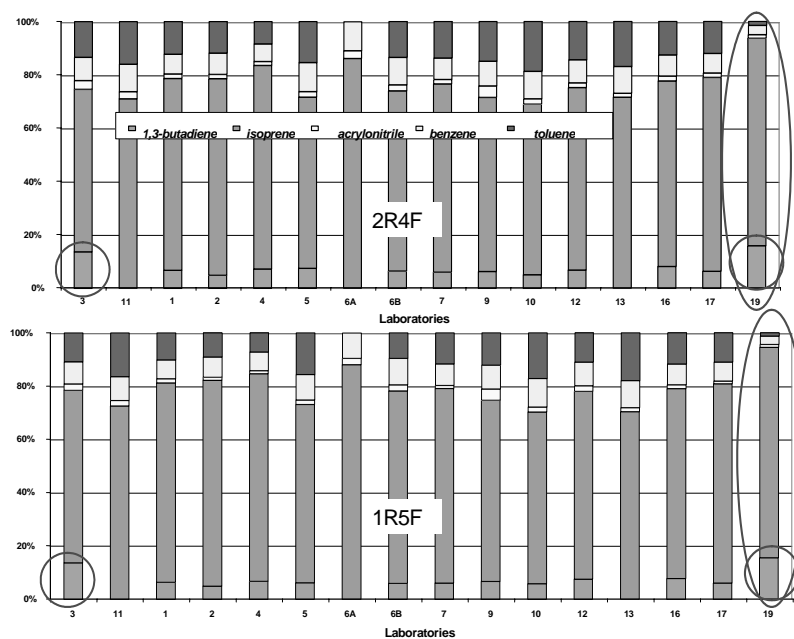
Figure A25. Ratio of patterns of selected volatiles (1,3-butadiene, isoprene, acrylonitrile, benzene, toluene) for 2R4F and 1R5F cigarettes. The yields of the five selected volatiles were summed together and expressed as individual percentages of the total yield as yield ratios. Major differences are highlighted.
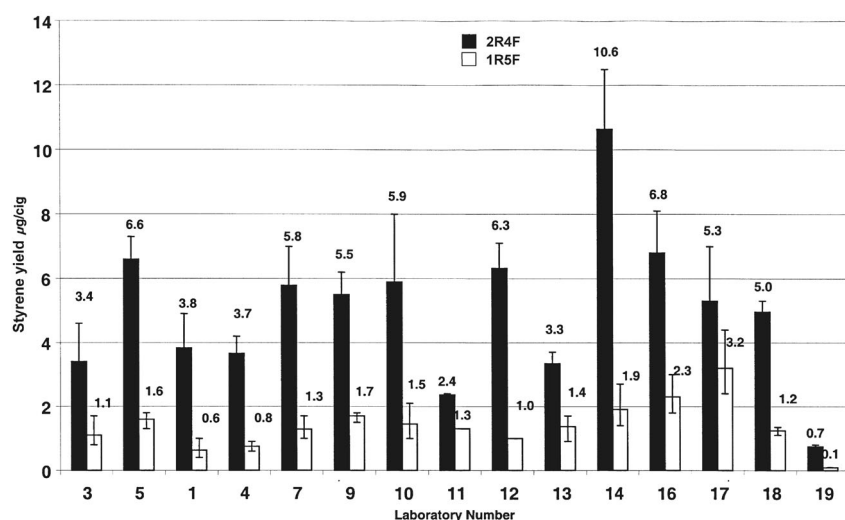
Appendix 40. Overview of styrene methodology

Table A21. Styrene methodology (EI = electron ionization; FID = flame ionization detection; GC-MS = gas chromatography-mass spectrometry; SIM = single ion monitoring)

Methodology	Laboratory							
	3	5	1	4	7	9	10	11
No. cigs per replicate	20	20	5-10	20	5	10	20	20
Type of smoking machine								
Linear or rotary	rotary	rotary	rotary	linear	linear	rotary	rotary	rotary
Make	Borgwaldt RM20	Borgwaldt RM200	Borgwaldt RM20	Borgwaldt RM20		Borgwaldt 20-port	Borgwaldt RM20CSR	Borgwaldt RM200
Trapping system								
Filter and diameter	CFP 92 mm	CFP 44 mm	CFP 92 mm	CFP 92 mm	CFP 44 mm	CFP 44 mm	CFP 92 mm	CFP 92 mm
Trapping	glass syringe	1 XAD-4 sorbent tube	1 Imp	2 Imp	1 Imp	3 Imp	2 Imp	1 Imp
Trapping solvent	styrene not extraxted from CFP	methanol	methanol	methanol	methanol	methanol	methanol	methanol
Extraction								
Filtration step	no	no	no	no	no	no	no	no
Clean-up								
Column clean-up step	no	no	no	no	no	no	no	no
Analytical equipment								
Description	GC	GC-MS	GC-MS	GC-MS	GC-MS	GC	GC-MS	GC
Detection system	FID	SIM	SIM	SIM	MS-EI	MS	SIM	FID

Methodology	Laboratory						
	12	13	14	16	17	18	19
No. cigs per replicate	10	20	5	20	10 or 20	5	10
Type of smoking machine							
Linear or rotary	rotary	rotary	linear	rotary	rotary	linear	linear
Make	Borgwaldt RM20/RM20H	Borgwaldt RM20	KC Automation 5-port	Borgwaldt	Borgwaldt RM20CSR	Internal	Borgwaldt RM200
Trapping system							
Filter and diameter	CFP 44 mm	CFP 92 mm	CFP 44 mm	CFP 92 mm	CFP 92 mm	CFP 44 mm	CFP 44 mm
Trapping	3 liquid impingers	2 Imp	2 Imp	2 Imp	1 Imp	1 Imp	no
Trapping solvent	methanol	methanol	methanol	methanol	methanol	methanol	methanol
Extraction							
Filtration step	yes	no	no	yes	yes	no	no
Clean-up							
Column clean-up step	no	no	no	no	no	no	no
Analytical equipment							
Description	GC-MS	GC-MS	GC-MS	GC-MS	GC-FID	GC-MS	GC-MS
Detection system	MS	SIM	SIM	MS ion trap positive EI	FID	SIM	MS

Appendix 41. Styrene yields

Figure A26. Styrene yields (µg/cig) for 2R4F and 1R5F cigarettes. Error bars give the maximum and minimum yields given by a laboratory expressed as an average of all replicates. Data at limit of quantification/detection (LOQ/LOD) was set at LOQ/2 just for this graphical analysis.

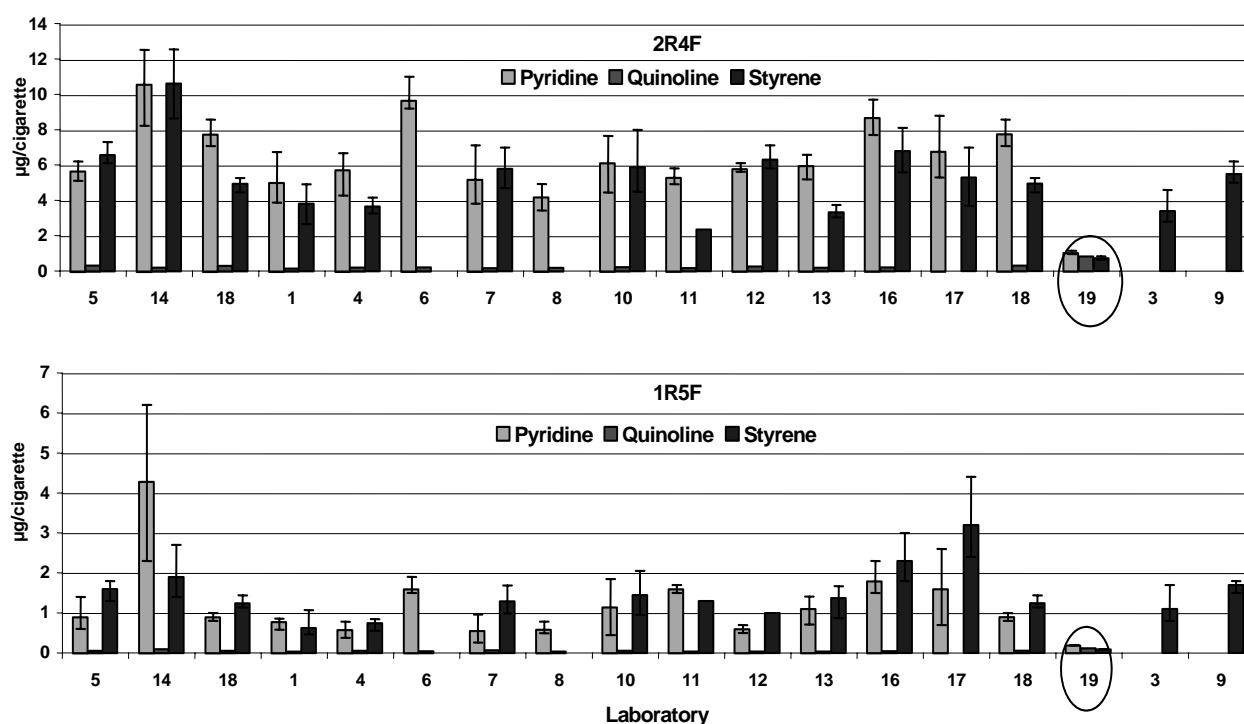Appendix 42. Overview of semi-volatiles methodology

Table A22. Overview of semi-volatiles methodology (EI = electron ionization; FID = flame ionization detection; MS = mass spectrometry; SIM = single ion monitoring)

Methodology	Laboratory							
	8	10	11	12	13	16	17	19
No. cigs per replicate	20	10	20	10	20	20	10 or 20	20
Type of smoking machine								
Linear or rotary	linear	rotary	rotary	rotary	rotary	rotary	rotary	linear
Make	Filtrona 20-prt	Borgwaldt RM20CSR	Borgwaldt RM200	Borgwaldt RM20/RM20H	Borgwaldt RM20	Borgwaldt	Borgwaldt RM20CSR	n/p
Trapping system								
Filter and diameter	CFP 44 mm	CFP 92 mm	CFP 92 mm	CFP 44 mm	CFP 92 mm	CFP 92 mm	CFP 92 mm	CFP 44 mm
Trapping	1 Imp	2 Imp	1 Imp	3 Imp	2 Imp	2 Imp	1 Imp	2 Imp
Solvent	methanol/ triethyl amine	methanol	methanol	methanol	methanol	methanol	methanol	methanol
Additional filtration	no	no	no	yes	no	yes	yes	yes
Analytical equipment								
Detection system	n/p	SIM	FID	MS	SIM	MS (ion trap)	FID	MS

Methodology	Laboratory						
	5	14	18	1	4	6	7
No. cigs per replicate	5	5	5	5-10	20	10	5
Type of smoking machine							
Linear or rotary	rotary	linear	linear	linear	rotary	rotary	linear
Make	Borgwaldt RM20	Cerulean ASM500	Internal	Cerulean SM450	Borgwaldt RM20	Borgwaldt RM20CSR	KC10
Trapping system							
Filter and diameter	CFP 44 mm	CFP 44 mm	CFP 44 mm	CFP 44 mm	CFP 92 mm	CFP 44 mm	CFP 44 mm
Trapping	1 XAD-4 sorbent tuibe	1 XAD-4 connected to CF unit	1 XAD-4 connected to CF unit	1 Imp	2 Imp	2 CFPs; acid pretreated	1 Imp
Solvent	methanol	methanol/hexane/ trimethyl amine	methanol	methanol	methanol	methanol	methanol/ triethyl amine
Additional filtration	no	no	no	no	no	no	no
Analytical equipment							
Detection system	SIM	SIM	SIM	SIM	SIM	SIM	MS-EI

Appendix 43. Yields of semi-volatiles (pyridine, quinoline and styrene)

Figure A27. Yields of semi-volatiles (µg/cig) (pyridine, quinoline and styrene) for 2R4F and 1R5F cigarettes. Error bars indicate the maximum and minimum yields given by a laboratory expressed as an average of all replicates

Appendix 44. Overview of TSNA methodology

Table A23. TSNA methodology (GC-TEA = gas chromatography-thermal energy analyser; MS = mass spectrometry)

Methodology	Laboratory							
	3	5	6A	6B	12	16	18	19
No. cigs per replicate	10	20	10	10	10	10	10	20
Type of smoking machine								
Linear or rotary	rotary	rotary	rotary	linear	rotary	rotary	linear	linear
Make	Borgwaldt RM20	Borgwaldt RM200	Borgwaldt RM20CSR		Borgwaldt RM20/RM20H	Borgwaldt RM20CS	Internal	n/p
Trapping system								
Filter and diameter	CFP 92 mm	CFP 92 mm	CFP 92 mm		CFP 44 mm	CFP 92 mm	CFP 44 mm	CFP 44 mm
Exctraction								
Solvent	sodium hydrogen phosphate/citric acid	dichloro methane	dichloro methane		dichloro methane	dichloro methane	dichloro methane	dichloro methane
Clean-up								
Column clean-up?	yes	yes	yes	yes	yes	yes	yes	yes
Analytical equipment								
Description	GC-TEA	GC-TEA	GC-TEA	GC-TEA	GC-TEA	GC-TEA	GC-TEA	GC-TEA
Detection system	TEA	TEA	TEA	TEA	TEA	TEA	TEA	TEA

Methodology	Laboratory					
	1	4	7	9	10	17
No. cigs per replicate	5-10	5	5	5-20	10	5
Type of smoking machine						
Linear or rotary	linear	rotary	linear	rotary	rotary	rotary
Make	Cerulean SM450	Borgwaldt RM200	n/p	Borgwaldt	Borgwaldt RM20CSR	Borgwaldt RM20CSR
Trapping system						
Filter and diameter	CFP 44 mm	CFP 44 mm	CFP 44 mm	CFP 44 mm	CFP 92 mm	CFP 44 mm
Exctraction						
Solvent	ammonium acetate	ammonium acetate	ammonium acetate	ammonium acetate	ammonium acetate	ammonium acetate
Clean-up						
Column clean-up?	no	no	no	no	no	no
Analytical equipment						
Description	LC-MS-MS	LC-MS-MS	LC-MS-MS	LC-MS-MS	LC-MS-MS	LC-MS-MS
Detection system	MS-MS	MS-MS	MS-MS	MS-MS	MS-MS	MS-MS

Appendix 45. Overview of TSNA yields (NNN = *N*-nitroso-nornicotine, NNK =
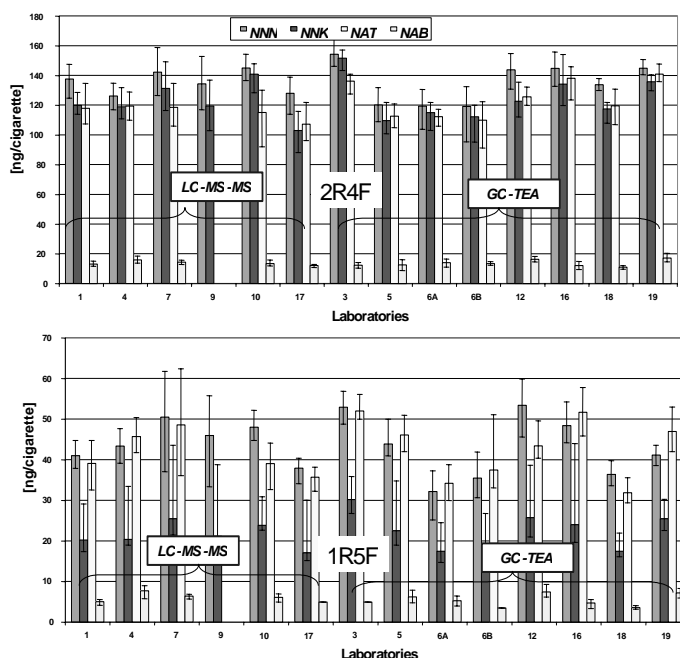
Figure A28. TSNA yields (ng/cig) (NNN, NNK, NAT, NAB) for 2R4F and 1R5F cigarettes. Error bars indicate the maximum and minimum yields given by a laboratory expressed as an average of all replicates (LC-MS-MS = liquid chromatography-mass spectrometry-mass spectrometry; GC-TEA = gas chromatography-thermal energy analyser).