

# Determination of Aromatic Amines in Cigarette Mainstream Smoke

## - The CORESTA 2007 Joint Experiment\*

by

Michael Intorp<sup>1</sup> and Steve Purkis<sup>2</sup>

<sup>1</sup>*Imperial Tobacco Group, Albert-Einstein-Ring 7, 22761 Hamburg, Germany  
Chairman of the CORESTA Special Analytes Task Force*

<sup>2</sup>*Imperial Tobacco Limited, P.O. Box 525, Southville, Bristol, BS99 1LQ, UK,  
Secretary of the CORESTA Special Analytes Task Force*

### SUMMARY

CORESTA joint experiment work in 2006 had compared data on a wide range of smoke constituents obtained from Kentucky reference cigarettes (1R5F and 2R4F), according to the existing methods used by participants. This work had identified that the methods used to determine aromatic amine yields in mainstream smoke would particularly benefit from further study to investigate the main weaknesses and influencing factors in their yield variability before progressing to full method standardisation. This report describes the output from a 2007 joint experiment to address these issues. Participating laboratories carried out experiments to investigate several factors that had been identified in the methodology as potential sources of variability. These were the amine derivative type, the derivatisation time and the point at which the addition of the internal standard for calibration occurred. A statistical assessment was made of their possible influence on aromatic amine smoke yields and yield reproducibility across different laboratories.

Results showed that aromatic amines again had poor between-laboratory yield reproducibility. The stage at which the internal standard was added to the smoke sample had the most significant effect on yields. The least variable data were obtained when it was added directly after extraction from the filter pad rather than later in the process. It also appeared beneficial to use at least two calibration standards (i.e., an aminonaphthalene and an aminobiphenyl) to minimise yield differences although this recommendation was not supported by statistically significant data.

Large differences in yields were not found when comparing the two studied derivatising agents especially when compared against the greater overall between-laboratory variability. Any differences between laboratories in total particulate matter and puff count at the smoke collection stage did not appear to significantly contribute to between-laboratory differences in yields.

It appeared that some laboratories had significantly improved their methodology since the last study although high values for the between-laboratory reproducibility in this study were still found. It may be that significant improvements in reproducibility may not be forthcoming for compounds such as the aromatic amines measured at low nanogram smoke yields.

Some important features that need to be controlled to minimise variability were identified in this study and will be incorporated within a collaborative study leading to a recommended method. Also, a wider range of product styles will need to be investigated, to determine the effects of differences in tobacco blends and product styles and the potential of greater product variability of commercial products. This should provide more robust estimates of within-laboratory repeatability and between-laboratory reproducibility. [Beitr. Tabakforsch. Int. 24 (2010) 78–92]

### ZUSAMMENFASSUNG

Im Rahmen einer CORESTA Studie wurden 2006 die Gehalte einer Vielzahl von Rauchinhaltsstoffen (Hoffmann Analyten) für die Kentucky Referenzzigaretten 1R5F und 2R4F verglichen, die bei Verwendung unterschiedlicher Methoden durch die teilnehmenden Labore gemessen

\*Received: 8<sup>th</sup> May 2009 – accepted: 11<sup>th</sup> December 2009

wurden. Die Bestimmung der aromatischen Amine im Hauptstromrauch von Zigaretten wurde dabei als Methode identifiziert, die besonders von der Durchführung einer Studie über methodische Schwächen und Faktoren, die Methodenstreuungen beeinflussen können, profitieren sollte.

Die vorliegende Arbeit beschreibt die Ergebnisse einer im Jahr 2007 durchgeführten Studie, in der diese Probleme behandelt werden. Die teilnehmenden Labore untersuchten verschiedene Faktoren, die als mögliche Ursachen von Methodenstreuungen vermutet wurden, speziell das Derivatisierungsreagens, die Reaktionszeit und die Zugabe des internen Standards. Der Einfluss der genannten Variablen auf die Gehalte an aromatischen Aminen und die Reproduzierbarkeit der Daten zwischen den beteiligten Laboren wurde statistisch bewertet.

Die Studie zeigte erneut große Unterschiede in den gefundenen Gehalten zwischen den Laboren und damit schlechte Reproduzierbarkeiten. Dabei hatte der Zusatz des internen Standards einen signifikanten Effekt. Die geringsten Streuungen wurden beobachtet, wenn der interne Standard direkt nach der Extraktion des berauchten Glasfaserfilters zugegeben wurde. Weiterhin erscheint es vorteilhaft, zwei unterschiedliche interne Standards (z.B. Aminonaphtalin und Aminobiphenyl) zu verwenden, obgleich die beobachteten Unterschiede im Gehalt statistisch nicht signifikant sind.

Die Verwendung zweier unterschiedlicher Reagenzien zur Derivatisierung der aromatischen Amine führte im Mittel zu keinen Unterschieden, insbesondere unter Berücksichtigung der Reproduzierbarkeit der Daten zwischen den Laboren.

Gleichwohl haben einige Labore ihre angewendeten Methoden deutlich verbessert, allerdings führte das nicht zur Verbesserung der Reproduzierbarkeiten, insbesondere für die Aminobiphenyle, was auch an den sehr niedrigen Gehalten dieser Substanzen im Hauptstromrauch von Zigaretten liegen könnte.

Im Rahmen der Studie konnten wichtige Parameter identifiziert werden, deren Festlegung bei der Durchführung einer zukünftigen Studie, mit dem Ziel eine einheitliche Methode festzulegen, von großem Nutzen ist. Weiterhin sind bei einer solchen Studie auch in einem größeren Umfang verschiedene Ausstattungen und Mischungstypen von kommerziellen Produkten zu berücksichtigen. Eine solche Studie sollte eine robustere Abschätzung der Wiederholbarkeiten innerhalb eines Labors wie auch der Reproduzierbarkeit zwischen den Laboren ermöglichen. [Beitr. Tabakforsch. Int. 24 (2010) 78–92]

## RESUME

En 2006, l'essai collectif CORESTA avait comparé des données provenant d'une large gamme de constituants de la fumée, obtenue à partir des cigarettes de référence Kentucky (1R5F and 2R4F), selon les méthodes existantes, propres à chaque participant à l'essai. Ce travail avait mis en évidence que les méthodes habituellement utilisées pour déterminer les taux d'amines aromatiques dans la fumée du courant principal pourraient bénéficier d'une étude plus poussée, afin d'examiner plus précisément les principales

faiblesses et les facteurs influents sur leur niveau de variabilité, avant de s'orienter vers une méthode de standardisation complète.

Ce rapport décrit les solutions, obtenues à la suite d'un essai collectif de 2007, pour répondre à ces problématiques. Les laboratoires participants ont effectué des expériences sur plusieurs facteurs qui avaient été identifiés dans la méthodologie comme des sources potentielles de variabilité. Ces facteurs sont le mode de dérivation de l'amine, le temps de dérivation et le moment auquel est effectué l'ajout du standard interne utilisé pour l'étalonnage. Une évaluation statistique a été menée, sur l'éventuelle influence de ces facteurs sur les taux d'amines aromatiques dans la fumée et sur la valeur de reproductibilité entre les différents laboratoires.

Les résultats ont montré une fois de plus que les amines aromatiques avaient une faible reproductibilité inter-laboratoire. L'étape durant laquelle le standard interne a été ajouté à l'échantillon de fumée est celle où l'effet sur les taux a été le plus significatif. Les résultats les moins variables ont été obtenus lorsque ce dernier a été ajouté directement après l'extraction du filtre, et non pas plus tardivement dans le processus. Bien que cela n'ait pas été confirmé par des données statistiquement significatives, il est aussi apparu avantageux d'utiliser au moins deux standards internes (c'est-à-dire un aminonaphtalène et un aminobiphenyl) afin de réduire au maximum les différences de teneurs.

La comparaison des deux agents de dérivation étudiés n'a mis en évidence aucunes grandes différences dans les taux, particulièrement après les avoir comparés à la plus grande variabilité inter-laboratoire dans son ensemble. Aucune différence entre les laboratoires sur la matière particulaire totale et le nombre de bouffée lors de l'étape de collecte de la fumée n'a semblé contribuer significativement aux différences inter-laboratoire des taux.

Il semble que quelques laboratoires aient significativement amélioré leur méthodologie depuis la dernière étude bien que de fortes valeurs de reproductibilité inter-laboratoire soient encore apparues lors de cette étude. Il se peut qu'aucune amélioration significative de la reproductibilité n'apparaisse pour des composés tels que les amines aromatiques, mesurés dans la fumée à des niveaux faibles de l'ordre du nanogramme.

Quelques paramètres importants, qui doivent être contrôlés pour réduire au minimum la variabilité, ont été identifiées dans cette étude et seront intégrées dans une étude collaborative menant à une méthode recommandée. De même, une gamme plus large de styles de produit devra être examinée, pour tenir compte des différents types de mélange et de la variabilité potentiellement plus grande des produits commerciaux. Cela devrait permettre des évaluations plus fiables des possibilités de répétition intra-laboratoire et de reproductibilité inter-laboratoire. [Beitr. Tabakforsch. Int. 24 (2010) 78–92]

## PARTICIPATING LABORATORIES

The authors wish to acknowledge the excellent contributions of each principal investigator without which this work would not have been completed.

Participating laboratories and institutions	Principal investigators
Altadis (France)	Valerie Troude, Sandrine Destruhaut
Arista (USA)	Richard Higby, Brad Norwood
Austria Tabak / Gallaher (Austria)	Jutta Müller
BAT - Souza Cruz	Waldenir Farias Braga
British American Tobacco (UK)	Helen Backshell, Jacqui Vella, Tina Mead
CNTC Beijing (China)	Jun Zhou
CNTC QSTC	Hongwei Hou
CNTC ZhengZhou (China)	Fuwei Xie
Filtrona Technology Centre (UK)	Joanne Walker
Imperial Tobacco Group / Reemtsma (Germany)	Michael Intorp, Steve Purkis
Japan Tobacco (Japan)	Takatsugu Hyodo
KT&G (Korea)	Hyo-Keun Kim
Labstat (Canada)	Mehran Sharifi, Pete Joza
Lorillard	Rob Stevens
Philip Morris Research Laboratories (Germany)	Joerg Diekmann, Klaus Rustemeier
Philip Morris International (Switzerland)	Michel Rotach
Reynolds Tobacco (USA)	Linda Crumpler
Sampoerna (Indonesia)	Bimo Haryo Progo
Consultant	Martin Whittaker (statistical interpretation)

## INTRODUCTION

Aromatic amines have been identified as biologically active substances present in cigarette smoke and are members of the so-called “Hoffmann Analyte” list, as described in the literature (1). Various methods of measurement of aromatic amines [1-aminonaphthalene (1-AN), 2-aminonaphthalene (2-AN), 3-aminobiphenyl (3-AB) and 4-aminobiphenyl (4-AB)] in cigarette smoke have also been described in the literature (2–9).

The CORESTA Special Analytes Task Force organised a joint experiment in 2005–2006 to compare data on a wide range of smoke constituents obtained from reference cigarettes according to the existing methods used by participants (10). This work had identified that aromatic amine yields had varied considerably between laboratories and to a greater extent than many other analytes on the “Hoffmann Analyte” list. It was therefore felt beneficial to investigate through further joint experiments the main weaknesses and influencing factors in their yield variability before proposing any particular methodology as a recommended method.

In the joint experiment described herein and carried out in 2007, participants provided aromatic amine yield data on reference cigarettes using their existing methods. All methods involved the derivatisation of aromatic amines after smoke extraction and prior to measurement. However, an experimental protocol was devised and followed in which laboratories carried out two sets of experiments to investigate three important factors that had been identified in the methodology as potential sources of variability i.e.,

the amine derivative type, the reaction time and the point at which the addition of the internal standard occurred.

A statistical assessment was made of the possible effects of these variables on aromatic amine smoke yields with respect to mean yield and reproducibility across different laboratories. It was also considered of special interest to assess the effects of these experimental factors on the power of discriminating between the two Kentucky Reference cigarettes (2R4F and 1R5F) among laboratories as described previously (10).

The possible effects of some other experimental variables on smoke yields were also evaluated, that is, differences in total particulate matter (TPM) of the smoke condensate produced by each laboratory that was subsequently analysed for aromatic amines; the influence of the different aromatic amines chosen as the internal calibration standard(s) and the derivatisation temperature. A comparison was made with previously reported results (10).

It was recognised that although a more intense regime may be introduced into the regulatory arena in the future, it was decided that the current ISO smoking regime (11) should be used for this joint experiment.

## EXPERIMENTAL

### *Overview of the methodology*

In simplistic terms, cigarettes were smoked and particulate phase was collected on a Cambridge filter pad (CFP). The pad was extracted with solvent and the extract in some cases was cleaned up on a cartridge column. The aromatic amines in the cleaned extract were derivatised before, in some cases, being put through a final clean-up procedure followed by quantitative measurement. A more detailed overview of the aromatic amine methodology, as applied in each participating laboratory in 2007, is described in the Appendices 1–3.

Eleven laboratories derivatised with pentafluoropropionic acid anhydride (PFPA), seven of these laboratories smoked on linear and four on rotary machines. A further seven laboratories used the heptafluorobutyric acid anhydride (HFBA) derivatisation method; two smoking on linear machines and five on rotary smoking machines.

The wide variation of clean-up and internal standard calibration procedures both of which might be major influencing factors on yield variation among laboratories are summarised and listed in Table 1.

Overall, it was observed that the applied PFPA methodology was more similar between laboratories in terms of extraction, clean up and derivatisation steps than the HFBA methodology.

### *Overview of the protocol*

The four aromatic amines 1-AN, 2-AN, 3-AB and 4-AB were the subject of this smoke study. Each laboratory roughly followed its own in-house methods analysing as many of them as was their normal practice. The main objective was to assess the possible effects of the main elements of the laboratory procedure on their yields. These elements were: the derivatising agents (PFPA versus

**Table 1. Examples of variations in methodologies used in laboratories**

	PFPA derivatisation method	HFBA derivatisation method
Derivatisation	Derivatisation was carried out under a wide range of conditions i.e. from 15 minutes to overnight at room temperature in dark conditions or overnight at refrigerated temperatures.	Derivatisation was carried out either for 30 minutes at 80 °C or for one hour at room temperature.
Clean up Columns (pre- derivatisation)	Laboratories used column cartridges containing various weights of Florisil (1-2g) with some adding sodium sulphate to eliminate water.	Laboratories applied different column cartridges for clean-up, for example, using either cation or anion exchange cartridges.
Elution from clean-up columns	Most laboratories eluted derivatives with either dichloromethane or with hexane/benzene/acetone (5:4:1) but with differing elution volumes.	Derivatives were eluted either stepwise, firstly with ammonium hydroxide/methanol then with toluene or one-step with toluene or one-step with hexane/ dichloromethane.
Internal standard	This was added at different stages of sample preparation either before dichloromethane partition or after first extraction with dichloromethane.	This was added at different stages of sample preparation either before extraction of Cambridge filter pad or after clean up but before derivatisation.

HFBA); the derivatisation time (30 minutes versus overnight); and the stage of addition of internal standard (after extraction from the Cambridge filter pad (CFP) versus directly before derivatisation).

Kentucky reference cigarettes (2R4F and 1R5F) were chosen for testing and were sourced from a single batch purchased from the University of Kentucky. In general, each laboratory performed one experiment similar to its normal procedure/regime and a second experiment by a different methodological regime.

The regime matrix was statistically designed to investigate three potentially important variables that have been identified in the methodology i.e., derivative type, derivatisation time, and the point at which the addition of the internal standard occurred. Practically, some laboratories found difficulty in conforming to some aspects of the requested study protocol and asked for their experiments to be modified. These changes led to an unbalanced final design across laboratories as described in Table 2.

**Table 2. Experimental Design as carried out by laboratories.**

The choice of clean-up procedure and the measurement/detection system was made entirely by the participating laboratories. The "Regime" numbers (1–6) given in brackets are referred to later in the text and in some Appendices.

Derivatisation time	Laboratory numbers - internal standard added		
	After extraction from filter pad	Directly before derivatisation	Before extraction from CFP
<i>PFPA derivatisation (Reaction at room temperature)</i>			
30 minutes	3, 7, 9, 17, 18, 19, 21, 22 [Regime 1]	3, 13, 16, 17, 18, 22 [Regime 2]	
Overnight	1, 9 [Regime 3]	1, 7, 13, 16, 19, 21 [Regime 4]	
<i>HFBA derivatisation (Reaction at 80 °C, lab 14 at room temp)</i>			
30 minutes	4, 5, 6, 10, 14, 23 [Regime 5]	4, 5, 10, 14, 23 [Regime 6]	6
Overnight	12		

Five replicates for each reference cigarette and experiment were generated in three independent smoking runs. It was requested that the five replicates should be run over 1–2 consecutive days and the two experiments should be run with a minimum of one week or longer between each experiment in order to incorporate within-laboratory variation into the resulting data; this procedure was achieved by most laboratories. The key elements studied by each laboratory are summarised in Table 2.

## RESULTS AND ANALYSIS

A summary of the full yield data for the four aromatic amines, received from the 18 participating laboratories for both 1R5F and 2R4F cigarettes, is given in Appendix 4. Some deviations from the original protocol made by various laboratories are described in Appendix 5.

### Product variability

TPM and puff count data were received from all 18 participating laboratories and are summarised in Table 3.

All laboratories smoked at least five cigarettes per replicate for 1R5F and 2R4F. Nine laboratories used "linear" and another nine laboratories used "rotary" smoking machines for smoke collection. Very similar TPM and puff count data were given across both smoking machine types. Overall, for TPM, a 4.5% coefficient of variation (CoV) for 2R4F and a 13.8% CoV for 1R5F cigarettes were obtained. The low puff count variability (CoV < 4%) indicated good adherence by the participating laboratories to conditioning standards (12).

Twelve of the 18 laboratories provided some weight data. The 1R5F cigarette had a mean weight of 0.849 g after conditioning and the 2R4F cigarette had a mean weight of 1.061 g after conditioning; these were very similar to weights measured in the previous study (10). The CoV in measured mean weights across each of these laboratories was only 0.8% indicating that the products had similar weight after standard ISO conditioning (12) in the different laboratories and weight is unlikely to be a major factor in any measured aromatic amine yield differences in this study.

**Table 3. TPM and Puff count data**

Parameter	Cigarette Type	Overall		Linear		Rotary	
		Mean	CoV %	Mean	CoV %	Mean	CoV %
TPM (mg/cig)	2R4F	10.87	4.5	10.84	4.4	10.91	4.1
	1R5F	2.24	13.8	2.03	8.3	2.45	11.4
Puff Count	2R4F	8.77	3.0	8.89	3.0	8.65	2.3
	1R5F	6.98	3.6	7.06	4.0	6.90	3.0

#### Statistical outliers and data removal

The full data-set consisted of over 4700 data points. It was assessed for irregularities and any difficulties reported by participants. Results of Experiment 2 from Laboratory 5 were excluded for the 2R4F sample because the yields were extremely low and this laboratory had recognised this as a problem during their work process. It can be noted that results from Laboratory 9 were reported only for analytes 2-AN and 4-AB. A statistical analysis of variance was carried out to identify the most extreme deviants among the replicate yields i.e., between-laboratory, within-laboratory, and between runs and this led to the removal of 14 individual replicates, just 0.3% of the full dataset as given in Appendix 5. This included four 1-AN, three 2-AN, three 3-AB and four 4-AB results. It is noted that 12 of these 14 were from Laboratory 10.

#### Data comparisons related to experimental design

Data were compared across the regimes (1–6) described in Table 2. Firstly, the effects of using the PFPA versus the HFBA derivatising agent were made by comparing data from Regimes 1 and 5 against 2 and 6. Secondly, the effects of a reaction time of 30 minutes versus overnight were investigated by comparing data from Regimes 1 and 3 against 2 and 4. Finally, the effects of adding the internal standard “After extraction” versus “Before derivatisation” were investigated by comparing data from Regimes 1, 3 and 5 against 2, 4 and 6.

Table 4 shows the mean yields for individual levels of each experimental factor for unmatched laboratories. The number of laboratories shown is the number of laboratory/experiment combinations. In this way, any particular laboratory can appear twice (once for each experiment) in the grouping of the testing regimes shown.

Based on analyses of variance of all the data, the mean yields were directionally consistent across the four aromatic amines and for both 2R4F and 1R5F reference cigarettes with

- the HFBA giving higher yields than the PFPA derivatisation agent,
- thirty minutes reaction time giving higher yields than overnight reaction although this includes data from both PFPA and HFBA derivatising agents which could possibly behave quite differently,
- addition of the internal standard at the “After extraction” stage giving higher yields than at the “Before derivatisation” stage.

However, these differences were statistically non-significant when compared with the variability in yields among laboratories.

Mean yield data were also compared for the nine matched laboratories (Numbers 1, 3, 4, 10, 14, 17, 18, 22 and 23). Again, there were significantly higher mean yields obtained when the internal standard was added “After extraction” rather than “Before derivatisation” for most analyte and cigarette type combinations.

**Table 4. Mean yields (ng/cig) for individual experimental factors (unmatched laboratories)**

Laboratory description	Experimental factors	1-AN		2-AN		3-AB		4-AB	
		2R4F	1R5F	2R4F	1R5F	2R4F	1R5F	2R4F	1R5F
15 labs using regimes 2, 4, 6	Internal standard addition - before derivatisation	10.2	3.0	6.2	1.7	1.8	0.64	1.3	0.47
19 labs using regimes 1, 3, 5	Internal standard addition - after extraction from CFP	11.5	3.3	7.3	2.1	2.1	0.68	1.4	0.48
26 labs using regimes 1, 2, 5, 6	Derivatisation time 30 minutes	10.9	3.0	7.3	2.0	1.9	0.68	1.4	0.51
8 labs using regimes 3, 4	Derivatisation time Overnight	10.0	2.4	6.8	1.8	2.0	0.54	1.3	0.39
22 labs using regimes 1, 2, 3, 4	Derivatisation agent PFPA	10.1	2.5	6.9	1.9	1.7	0.55	1.3	0.43
12 labs using regimes 5, 6	Derivatisation agent HFBA	10.9	2.9	7.3	2.0	2.2	0.67	1.4	0.47
Matched laboratories (numbers 1, 3, 4, 10, 14, 17, 18, 22, 23)	Internal standard addition - after extraction from CFP	11.9	3.8	7.5	2.2	2.0	0.79	1.5	0.61
	Internal standard addition - before derivatisation	10.4	3.4	6.3	1.9	1.9	0.80	1.4	0.61

### Discrimination between 2R4F and 1R5F cigarettes

A similar statistical assessment was made as described in the 2007 CORESTA report, on the basis that the two reference cigarettes were tested by different laboratories, together with the assumptions that each analyte was roughly correlated with the Nicotine Free Dry Particulate Matter (NFDPM) for these two reference cigarettes. Their NFDPM yields are distinctly different i.e., 1.7 and 9 mg/cig. The equations used for this assessment are shown in Appendix 6 whilst a more detailed description of this analysis can be found in the previous study report (10).

In the graph in Appendix 6, the vertical axis defines the smallest difference between the mean yields for 2R4F and 1R5F that can be distinguished, 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 the studied analytes on one graph. In this case, 'R' is an estimate of the between-laboratory reproducibility although 'R' can only be properly applied when laboratories use the same standardised methods in each laboratory.

Points on the vertical axis which are greater or equal to 100 indicate that for these analytes it would not be possible to distinguish, with 95% statistical confidence, between 1R5F and 2R4F cigarettes using the current range of methodologies when comparing between-laboratory data. For these analytes there would appear to be the greatest need to further investigate some of the weakness of the methodologies currently run in the laboratories before progressing to a full collaborative study using one standard method. Conversely, for analytes whose points on the Y axis are less than 100, the 1R5F and 2R4F samples would be distinguished (with 95% confidence). However, even for those analytes with values substantially less than 100 there is still a need to progress to a full collaborative study using the same methodology across all laboratories to determine proper within- and between-laboratory variability data as required for inclusion in any ISO standardised method.

In this analysis, the power of discrimination of the current methodologies between 1R5F and 2R4F cigarettes was made for each of the different experimental regimes, as shown graphically in Appendix 6 and also grouped together in Table 5.

Data shown in Appendix 6 suggested a greater discrimination when the internal standard was added "After extraction" compared to "Before derivatisation" (regimes 1:2, 3:4 and 5:6). Differences between overnight and 30-minute derivatisation (regimes 3:1 and 4:2) were less clear as were those between the different derivatising agents HFBA and PFPA (regimes 5:1 and 6:2). This is to some extent due to results being confounded by mismatching of laboratories across the six regimes. The least desirable factor combination would appear to be 'PFPA derivatising agent for 30

minutes derivatisation time with the internal standard added before derivatisation' (i.e., testing regime 2) for which discrimination between the two samples was particularly poor for analytes 3-AB and 4-AB.

### Relationship between TPM and aromatic amine yields

TPM was positively correlated with the amine yields, the linear regressions being statistically significant for all four analytes and both 1R5F and 2R4F with the sole exception of 1-AN for the 2R4F cigarette which was not significant. As examples, the relationships between 1-amino-naphthalene and TPM yields are shown graphically in Appendix 7.

In view of these correlations it was decided to obtain estimates of %CoV[R(2R4F–1R5F)] with TPM as a co-variate in an attempt to 'sharpen' the comparison across the testing regimes. As expected the resulting variability among laboratories was reduced for some analytes and samples, but relative values of %CoV[R(2R4F–1R5F)] across regimes were not substantially changed and are not included in this report.

The plots of the yields of four aromatic amines versus TPM were also inspected for correlated extremes in the mean yields and TPM. It was thought that, if this occurred consistently for a particular laboratory, over all or most analytes and for both 1R5F and 2R4F, it could indicate a smoking machine problem but this effect was not found.

In summary, data from this study suggest that differences in the smoke collection stage are not a major factor in laboratory differences in mean aromatic amine yields in cigarette smoke.

### Choice of internal standards for calibration

Across the 18 laboratories, different procedures were followed when applying the internal standard(s). For example, Laboratory 1 used just one deuterated internal standard, 4-AB *d*9, for calibrating the yields for all four analytes, whereas Laboratory 21 used three deuterated internal standards 1-AN *d*7, 2-AN *d*7 and 4-AB *d*9 with the 3-AB yields being calibrated indirectly against the internal standard for 4-AB *d*9. A summary of the internal standards is given in Table 6.

To examine the data for possible effects due to using internal standards for direct and indirect calibration, the plots shown in Appendices 8–10 were produced. The legend in each plot denotes which internal standard has been used to determine aromatic amine yields. In some cases, yields were measured directly from the corresponding standard, for example, 1-AN *d*7 to measure 1-AN and in other cases indirectly; for example, 2-AN *d*7 to measure 1-AN. None of the laboratories used the deuterated 3-AB as the internal standard.

These plots were used to investigate whether the mean yields relating to a calibration using a direct internal standard were predominantly higher, lower or more consistently grouped than those relating to indirect calibration. Although no consistent effects were apparent, there was some indication that using the deuterated 4-AB standard for 1-AN and 2-AN gives some of the most extreme results and using the deuterated 1-AN standard to calibrate for 4-AB

**Table 5. Discrimination between 2R4F and 1R5F cigarettes**

Experimental Regimes	%CoV R (2R4F – 1R5F)			
	1-AN	2-AN	3-AB	4-AB
1, 3 and 5	72	76	78	88
1, 2, 3, 4, 5 and 6	87	91	106	113

**Table 6. Internal standards normally used in participating laboratories**

Laboratory	1-AN d7	2-AN d7	4-AB d9
1	...	...	yes
3	...	yes	yes
4	yes	yes	yes
5	...	yes	yes
6	yes	yes	yes
7	...	...	yes
9	...	yes	yes
10	yes	yes	yes
12	yes	yes	yes
13	...	...	yes
14	yes	yes	yes
16	...	...	yes
17	...	yes	yes
18	yes	...	...
19	...	...	yes
21	yes	yes	yes
22	yes	...	...
23	...	yes	yes

for 1R5F also gives high values as shown in Appendices 8–10. The standards used to calibrate for 1-AN, 2-AN and 3-AB yields for 1R5F cigarettes gave no discernible effects and results are not shown in this report.

#### *Comparison with data from the last CORESTA study*

Appendix 11 relates to a comparison of data from the 11 laboratories with no missing results. Good agreement is shown for the difference in mean yields between 2R4F and 1R5F cigarettes. The %CoV[R(2R4F–1R5F)] value for analyte 4-AB in the current study appears to be much lower than found in the previous study indicating that, overall, the laboratories have improved their power of discriminating between cigarettes. In the previous study some laboratories failed to measure 3-AB and 4-AB for sample 1R5F but must have subsequently improved their limits of detection and quantification.

Regimes 1 and 5 appear to have the optimal studied conditions incorporating PFPA and HFBA derivatisation, respectively. Table 7 shows the mean yield, 'r' and 'R' from the previous study for the complete data set and for the current study for each of these two regimes for all laboratories. Whilst these comparisons derive from different groups of laboratories, it is noted that the mean yields are in broad agreement but the 'R' values are always higher from the previous study than for each of regimes 1 and 5 from the current study. This appears to indicate that, overall, laboratories are acquiring more measurement expertise and improving after participating in these collaborative studies.

## CONCLUSIONS AND RECOMMENDATIONS

Some laboratories have significantly improved their methodology since the 2006 Joint Experiment.

The differences in TPM and puff count were found to be small and so the smoking and smoke collection steps are unlikely to significantly contribute to differences in aromatic amine yields.

Three factors had been identified by the Task Force in the methodology as potential and important sources of variability and were particularly investigated. These were: the amine derivative type, the reaction time, and the point at which the addition of the internal standard occurred.

Large differences in yields were not found when comparing the two derivatising agents (PFPA and HFBA). However, using PFPA as the derivatising agent may be easier to take through the standardisation process, rather than HFBA, due to less difference in the methodology steps between laboratories. Although not supported by statistically significant data, the use of two internal standards for calibration, that is, both an aminonaphthalene and an aminobiphenyl would seem a wise choice within any proposed methodology.

The least variable experimental conditions were found to be the shorter 30 minute reaction time with the internal standard added after smoke extraction from the filter pad and before derivatisation with either PFPA or HFBA

**Table 7. Estimates of mean yield (ng/cig), repeatability (r) and reproducibility (R) - CORESTA studies in 2006 and 2007**

Study	Statistic <sup>a</sup>	1-AN		2-AN		3-AB		4-AB	
		2R4F	1R5F	2R4F	1R5F	2R4F	1R5F	2R4F	1R5F
2006	Mean	12.04	3.66	8.31	2.40	2.16	0.84	1.50	0.63
	r	1.10	0.32	0.70	0.24	0.22	0.13	0.31	0.14
	R	9.75	4.67	6.66	2.41	1.90	1.12	1.53	0.98
2007 PFPA Regime 1	Mean	12.12	3.45	7.76	2.21	1.93	0.67	1.39	0.51
	r	1.28	0.40	0.58	0.19	0.15	0.06	0.09	0.04
	R	7.99	2.73	5.26	1.80	1.06	0.77	1.11	0.69
2007 HFBA Regime 5	Mean	11.90	3.76	7.68	2.21	2.21	0.78	1.51	0.56
	r	1.51	0.52	0.93	0.23	0.23	0.10	0.17	0.08
	R	8.56	3.08	6.29	1.68	1.70	0.43	0.85	0.49

<sup>a</sup>  $r = 2 \times \{2 \times \text{Var}(\text{Rep})/5\}^{1/2}$  - where Var(Rep) denotes the variance between replicate yields nested within laboratories and runs,  $R = 2 \times \{2 \times [\text{Var}(\text{Lab}+\text{Run})+(\text{Var}(\text{Rep})/5)]\}^{1/2}$  - where Var(Lab+Run) denotes the sum of the variances between laboratories and between runs tested within laboratories.

derivatising agents, rather than later in the work-up procedure. Under these conditions it was possible to consistently differentiate between-laboratory data between 1R5F and 2R4F cigarettes with statistical confidence for all four aromatic amines.

Even so, the between-laboratory variability for aromatic amines using the various non-standardised methodologies currently used at the participating laboratories was high with data in the range of mean  $\pm$  (60–135%) across the four analytes.

In the future, the CORESTA Task Force will work towards a recommended method using the preferred parameters found in this study. Using such a uniform method should further reduce measurement variability within any proposed collaborative study which should also investigate a wider range of product/blend styles to determine any potentially greater product variability of commercial products.

## REFERENCES

- Hoffmann, D. and I. Hoffmann: The changing cigarette: Chemical studies and bioassays; *in*: Risks associated with smoking cigarettes with low machine-measured yields of tar and nicotine; edited by DHSS, Smoking and Tobacco Control Monograph 13, NCI, NIH, Bethesda 2001, Chapter 5.
- Hoffmann, D., Y. Masuda, and E.L. Wynder:  $\alpha$ -Naphthylamine and  $\beta$ -naphthylamine in cigarette smoke; *Nature* 221 (1969) 254–256.
- Patrianakos, C. and D. Hoffmann: Chemical studies on tobacco smoke: LXIV. On the analysis of aromatic amines in cigarette smoke; *J. Anal. Toxicol.* 3 (1979) 50.
- Pieraccini, G., F. Luceri, and G. Moneti. New gas-chromatographic/mass-spectrometric method for the quantitative analysis of primary amines in main and sidestream cigarette smoke; *Rapid Commun. Mass Spectrom.* 6 (1992) 406–409.
- Luceri, F., G. Pieraccini, G. Moneti, and P. Dolora: Primary aromatic amines from side-stream cigarette smoke are common contaminants of indoor air; *Toxicol. Ind. Health* 9(3) (1993) 405.
- Health Canada Method T102: Determination of 1- and 2- aminonaphthalene and 3- and 4- aminobiphenyl in mainstream tobacco smoke (accessed July 2010) [http://www.hc-sc.gc.ca/hc-ps/alt\\_formats/hecs-sesc/pdf/tobac-tabac/legislation/reg/indust/method/\\_main-principal/amino-eng.pdf](http://www.hc-sc.gc.ca/hc-ps/alt_formats/hecs-sesc/pdf/tobac-tabac/legislation/reg/indust/method/_main-principal/amino-eng.pdf)
- Forehand, J.B., G.L. Dolly, and S.C. Moldoveanu: Analysis of polycyclic aromatic hydrocarbons, phenols and aromatic amines in particulate phase cigarette smoke using simultaneous distillation and extraction as a sole sample clean-up step; *J. Chromatogr. A* 898 (2000) 111–124.
- Smith, C.J., G.L. Dooly, and S.C. Moldoveanu: New technique using solid-phase extraction for the analysis of aromatic amines in mainstream cigarette smoke; *J. Chromatogr. A* 999 (2003) 99–107.
- Stabbert, R., K.-H. Schäfer, C. Biefel, and K. Rustemeier: Analysis of aromatic amines in cigarette smoke; *Rapid Commun. Mass Spectrom.* 17 (2003) 2125–2132.
- Intorp M., S. Purkis, M. Whittaker, and W. Wright: Determination of “Hoffmann analytes” in cigarette mainstream smoke. The CORESTA 2006 Joint Experiment; *Beiträge Tabakforsch. Int.* 23(4) (2009) 161–202.
- ISO Standard 3308: Routine analytical cigarette smoking machine – Definitions and standard conditions; International Standardisation Organisation, Geneva, 2000.
- ISO Standard 3402: Tobacco and tobacco products – Atmosphere for conditioning and testing; International Standardisation Organisation, Geneva, 1999.

*Address for correspondence:*

Steve Purkis  
Imperial Tobacco Limited  
PO Box 525  
Southville  
Bristol BS99 1LQ, UK  
E-mail: [steve.purkis@uk.imptob.com](mailto:steve.purkis@uk.imptob.com)



## APPENDIX

### Appendix 1. Methodologies used by participating laboratories – smoking and extraction steps

Lab No.	Cig/replicate		Smoking machine		Cambridge filter pad extraction		
	1R5F	2R4F	Linear	Rotary	Pad size (mm)	Solvent	Volume/time
1	5	5	Cerulean SM500	...	44	5% hydrochloric acid	25 mL/30 min
3	10	10	...	Borgwaldt RM20	92	5% hydrochloric acid	100 mL/30 min
4	10	10	...	Borgwaldt	92	5% hydrochloric acid	100 mL/60 min
5	20	20	...	Borgwaldt	92	5% hydrochloric acid	100 mL/30 min
6	5	5	Cerulean SM450	...	44	5% hydrochloric acid	10 mL/30 min
7	5	5	HawkTech	...	44	5% hydrochloric acid	25 mL/30 min
9	15	5	...	Borgwaldt RM20H	44	dilute hydrochloric acid (x2)	Σ20 mL/Σ 30 min
10	10	10	...	Borgwaldt	92	0.25 M hydrochloric acid I	60 mL/30 min
12	10	10	...	Borgwaldt	44	dilute hydrochloric acid/methanol	40 mL/10 min
13	10	10	Cerulean SM450	...	44	5% hydrochloric acid	100 mL/30 min
14	5	5	Cerulean SM500	...	44	hexane/trimethylamine	not given
16	5	5	...	Borgwaldt	92	5% hydrochloric acid	100 mL/30 min
17	5	5	Cerulean	...	44	5% hydrochloric acid	25 mL/30 min
18	15	15	Cerulean	...	44	5% hydrochloric acid	25 mL/30 min
19	10	10	Cerulean SM450	...	44	5% hydrochloric acid	100 mL/60 min
21	10	5	Cerulean SM450	...	44	5% hydrochloric acid	25 mL/30 min
22	20	20	...	Borgwaldt RM200	92	5% hydrochloric acid	200 mL/30 min
23	10	10	...	Borgwaldt RM200	92	dichloromethane	Σ100 mL/Σ60 min

### Appendix 2. Methodologies used by participating laboratories – pre-derivatisation steps (SPE = solid phase extraction)

Lab No.	Derivative	Clean-up before derivatisation			
		Cartridge	Wash	Eluant	Basification step
1	PFFA	no	dichloromethane	no	pH > 12 to dichloromethane
3	PFFA	no	dichloromethane/ cyclohexane	no	pH = 11 to hexane
7	PFFA	no	dichloromethane	no	pH = 10 to dichloromethane
9	PFFA	no	no	no	pH = basic to hexane
13	PFFA	no	dichloromethane	no	pH > 12 to hexane
16	PFFA	no	dichloromethane	no	pH > 12 to hexane/benzene/acetone
17	PFFA	no	dichloromethane	no	pH = basic to hexane
18	PFFA	no	dichloromethane	no	pH = 13 to hexane
19	PFFA	no	dichloromethane	no	pH = 12 to hexane
21	PFFA	no	no	no	pH > 10 to dichloromethane
22	PFFA	no	dichloromethane	no	pH = 12 to hexane
4	HFBA	SPE	hydrochloric acid/methanol	ammonium hydroxide/ methanol	pH = 11 SPE to toluene
5	HFBA	SPE	hydrochloric acid/methanol	ammonium hydroxide/ methanol	pH = 11 SPE to toluene
6	HFBA	SPE	hydrochloric acid/methanol	ammonium hydroxide/ methanol	pH = basic SPE to toluene
10	HFBA	no	no	no	pH = 11 SPE to methanol/water
12	HFBA	no	no	no	pH > 12 SPE to methanol/water
14	HFBA	SPE	hexane	hexane/ dichloromethane	no
23	HFBA	no	no	no	derivatisation of dichloromethane extract

### Appendix 3. Summary of methodologies used by participating laboratories – post-derivatisation steps

Lab No.	Derivatised material	Final clean-up					Detection system <sup>b</sup>
		Absorption solvent	Absorbent <sup>a</sup>	Clean-up	Elution solvent/final solvent	Final volume	
1	All	dichloromethane	florisil	manual	dichloromethane	1 mL	GC/MS - EI
3	All	hexane/benzene/acetone	florisil	manual	hexane/benzene/acetone	not stated	GC/MS - EI
7	All	dichloromethane	florisil	manual	dichloromethane	1 mL	GC/MS - NCI
9	All	...	...	...	hexane	2 mL	GC/MS/MS NCI
13	All	hexane/benzene/acetone	florisil	manual	hexane/benzene/acetone	not stated	GC/MS - EI
16	...	...	florisil	...	hexane/benzene/acetone	not stated	GC/MS
17	All	...	...	...	hexane	0.75 mL	GC/MS - NCI
18	All	hexane	florisil	manual	hexane/benzene/acetone	1 mL	GC/MS - EI
19	All	hexane	florisil	manual	dichloromethane	1 mL	GC/MS - EI
21	All	not stated	florisil	manual	dichloromethane	1–2 mL	GC/MS - NCI
22	All	not stated	florisil	automated	hexane/benzene/acetone	0.5 mL	GC/MS - EI
4	All	toluene	florisil	manual	toluene	1.5 mL	GC/MS - NCI
5	All	...	...	...	toluene	not stated	GC/MS - EI
6	Aliquot	...	...	...	toluene	not stated	GC/MS - NCI
10	Aliquot	...	...	...	...	...	GC/MS - NCI
12	All	n-pentane	florisil	manual	hexane/acetone	0.5 mL	GC/MS - EI
14	All	hexane/dichloromethane	SAX	manual	hexane/dichloromethane	1 mL	GC/MS - EI
23	Aliquot	dichloromethane	silica gel	...	dichloromethane	not stated	GC/MS - NCI

<sup>a</sup> Florisil and SAX are specific adsorbent materials

<sup>b</sup> GC/MS = gas chromatography mass spectrometry ; NCI = negative chemical ionisation; EI = electron ionisation

### Appendix 4. Mean yields across laboratories (These data include all data from each experiment across all replicates before any statistical outliers were removed; SD = standard deviation.)

Lab No.	1-Aminonaphthalene (ng/cig)				2-Aminonaphthalene (ng/cig)				3-Aminobiphenyl (ng/cig)				4-Aminobiphenyl (ng/cig)			
	2R4F		1R5F		2R4F		1R5F		2R4F		1R5F		2R4F		1R5F	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	7.74	1.70	2.24	0.45	4.97	1.05	1.55	0.31	1.52	0.30	0.57	0.15	1.15	0.22	0.44	0.08
3	13.36	1.36	3.88	0.30	8.71	0.90	2.59	0.20	2.02	0.19	0.68	0.05	1.72	0.22	0.70	0.11
4	12.46	0.99	4.25	0.35	8.45	0.86	2.76	0.25	2.17	0.19	0.81	0.07	1.60	0.15	0.61	0.05
5	7.00	4.39	2.85	1.53	5.37	3.40	1.79	1.07	1.40	0.66	0.54	0.26	0.99	0.49	0.38	0.19
6	10.25	0.88	2.34	0.20	7.82	0.87	1.99	0.24	1.95	0.24	0.59	0.07	1.44	0.21	0.44	0.07
7	9.79	0.81	2.30	0.26	5.43	0.42	1.29	0.13	1.90	0.19	0.50	0.05	1.08	0.10	0.31	0.03
9	...	...	...	...	6.42	0.42	2.01	0.21	...	...	...	...	1.03	0.10	0.39	0.04
10	15.71	3.57	5.33	1.17	8.98	1.67	2.86	0.66	2.47	0.65	0.92	0.24	1.74	0.44	0.64	0.15
12	11.72	0.49	3.35	0.18	8.09	0.39	2.19	0.09	3.09	0.19	0.82	0.08	1.62	0.08	0.57	0.05
13	6.11	1.63	1.72	0.65	3.13	0.54	0.97	0.30	0.82	0.11	0.35	0.09	0.68	0.22	0.23	0.08
14	7.43	0.79	2.52	0.38	3.03	0.40	1.45	0.24	1.41	0.22	0.86	0.22	1.08	0.33	0.66	0.32
16	17.08	2.48	4.31	0.83	10.57	1.21	2.47	0.47	2.31	0.31	0.62	0.11	1.80	0.23	0.47	0.08
17	9.83	0.80	2.64	0.42	6.87	0.62	2.19	0.30	1.59	0.22	0.56	0.10	1.04	0.14	0.39	0.07
18	10.97	1.41	3.34	0.31	6.14	0.67	1.50	0.18	1.36	0.14	0.37	0.04	1.05	0.13	0.30	0.04
19	11.20	1.19	3.36	0.55	8.36	0.77	2.48	0.38	1.51	0.11	0.52	0.09	1.05	0.07	0.39	0.05
21	9.38	1.07	2.14	0.24	5.88	0.64	1.47	0.16	1.79	0.21	0.55	0.05	1.30	0.14	0.40	0.04
22	9.93	1.17	3.98	0.28	7.29	1.10	2.32	0.48	2.38	0.18	1.51	0.23	1.93	0.14	1.21	0.17
23	13.44	1.47	4.32	0.29	7.60	1.06	1.64	0.23	2.54	0.32	0.89	0.09	1.63	0.17	0.54	0.06

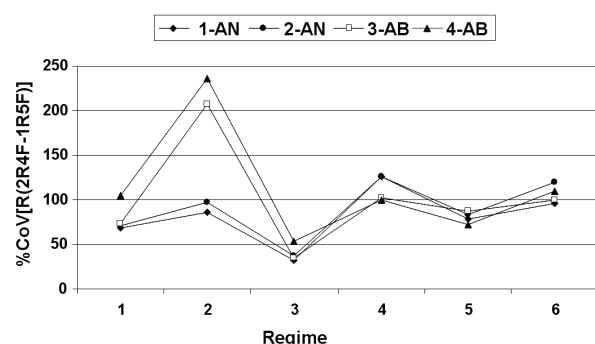
## Appendix 5. Deviations from protocol and statistical outliers

- Laboratory 4 sourced 2R4F cigarettes in-house rather than from prescribed batch for the 3rd smoking run. The in-house method was changed over to the HFBA derivative after the protocol design but prior to this study.
- Laboratory 5 indicated that they had problems with the internal standard addition in their Experiment 2.
- Laboratory 6 could not follow the proposed experiments in a timely manner with the necessary resource. For their experiments, they added the internal standard prior to extraction for Experiment 2 and added to extracted material directly after extraction and filtration for Experiment 1. The advantage of their method is that adding the internal standard before further sample processing means they do not need to perform quantitative solution transfers so allowing automation in the sample processing.
- Laboratory 12 sourced 1R5F and 2R4F in-house rather than from the prescribed batch. Experiment 1 was analysed in three different weeks although Experiment 2 were smoked in three smoking runs on three days in one week.
- Laboratory 14 carried out derivatisation at room temperature rather than at 80 °C. Samples were frozen for several days, after derivatisation but before measurement.
- Laboratory 16 did not prepare samples a minimum one week apart due to time constraints. However, different technicians were used with each set in order to mimic the time differences. Also, the same smoked sample was used for both Experiments 1 and 2 by splitting the sample immediately after the extraction of the pad and processing the split samples independently.
- Laboratory 23 could not return all the results performed in one smoking run per week due to the short available time-span. So, all experiments were carried out in the same week.

## Statistical Outliers

Analyte	Experiment	Sample	Run	Replicate	Laboratory	Yield (ng/cig)
1-AN	1	2R4F	1	2	10	12.12
1-AN	1	2R4F	1	5	10	26.85
1-AN	2	2R4F	3	3	10	22.9
1-AN	2	1R5F	1	5	10	8.97
2-AN	2	2R4F	3	3	10	14.78
2-AN	1	1R5F	1	5	10	3.86
2-AN	2	1R5F	1	5	10	4.99
3-AB	2	2R4F	3	3	10	5.13
3-AB	1	1R5F	1	5	10	1.34
3-AB	2	1R5F	1	5	10	1.55
4-AB	1	2R4F	2	1	4	1.67
4-AB	2	2R4F	3	3	10	3.68
4-AB	2	1R5F	1	5	13	0.54
4-AB	2	1R5F	1	5	10	1.10

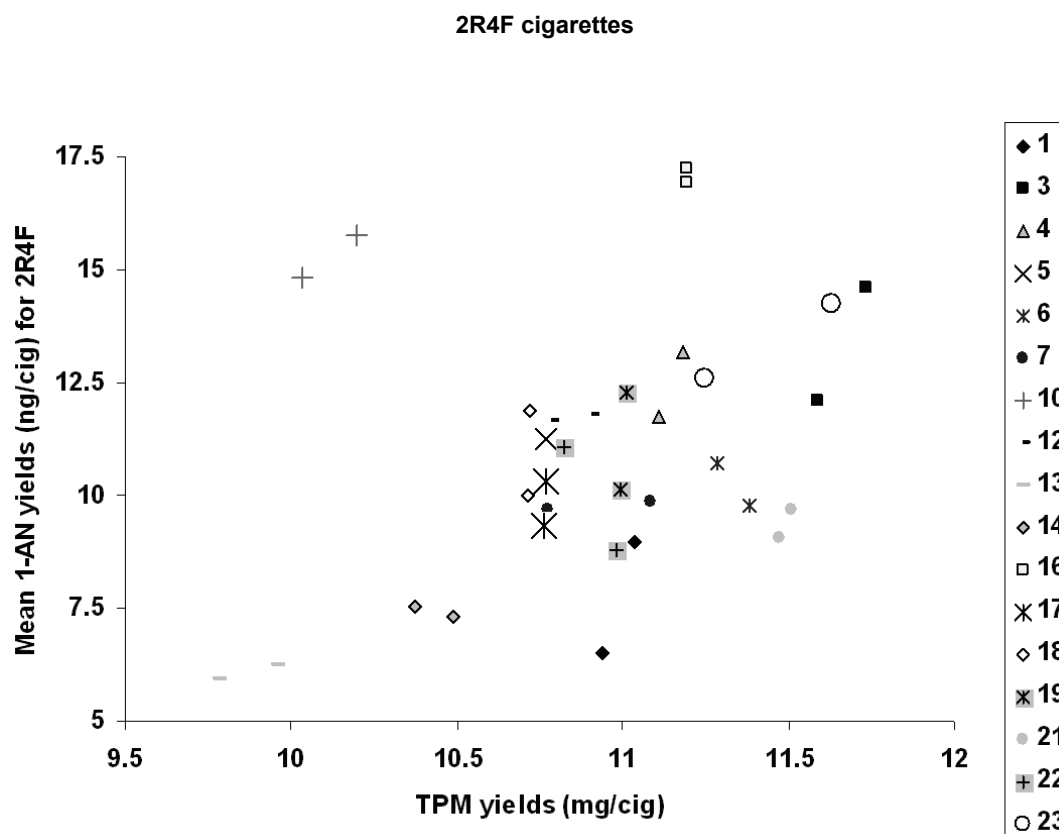
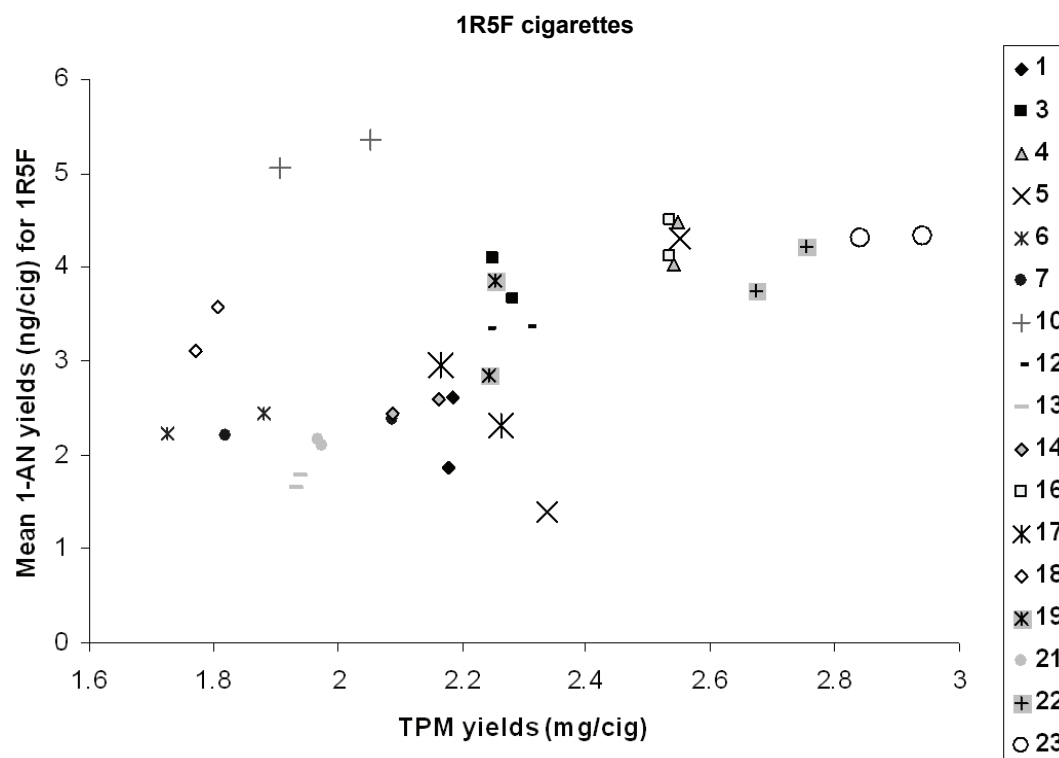
## Appendix 6. Discrimination between 2R4F and 1R5F cigarettes by different experimental regimes



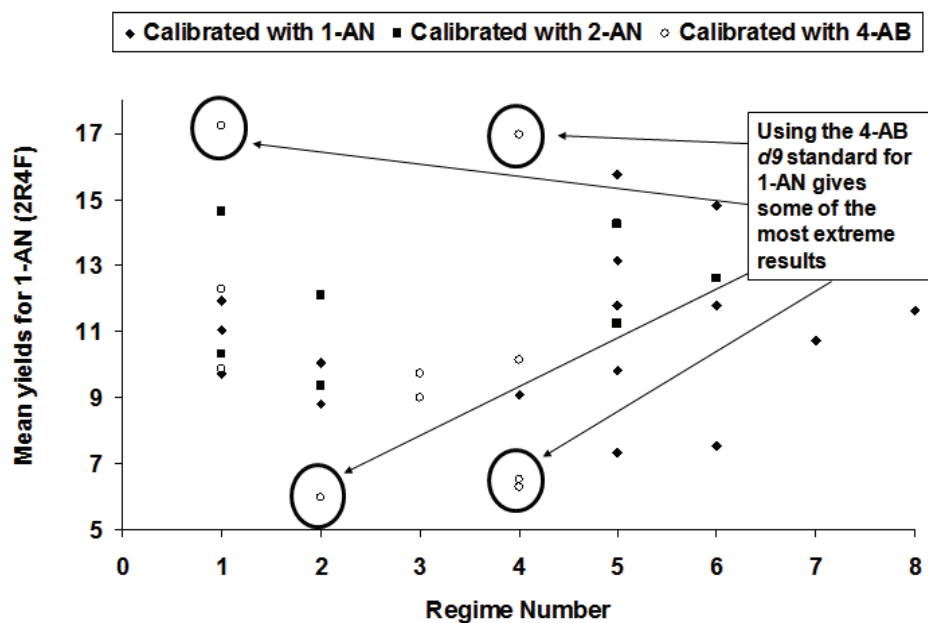
Where  $\%CoV [R(2R4F - 1R5F)] = \frac{100 \times R(2R4F - 1R5F)}{\text{Mean}(2R4F - 1R5F)}$

- $R(2R4F - 1R5F) = 2 \{ [SD(L_{2R4F})^2 + SD(L_{1R5F})^2] + [SD(r_{2R4F})^2 + SD(r_{1R5F})^2]/5 \}^{1/2}$
- $SD(L_{2R4F})$  and  $SD(L_{1R5F})$  are the standard deviations among laboratories for 2R4F and 1R5F.
- $SD(r_{2R4F})$  and  $SD(r_{1R5F})$  are the standard deviations between replicates, pooled over laboratories and experiments.
- $\text{Mean}(2R4F - 1R5F)$  is the mean difference between 2R4F and 1R5F, averaged across laboratories.
- 1-AN = 1-aminonaphthalene; 2-AN = 2-aminonaphthalene; 3-AB = 3-aminobiphenyl; 4-AB = 4-amino-biphenyl

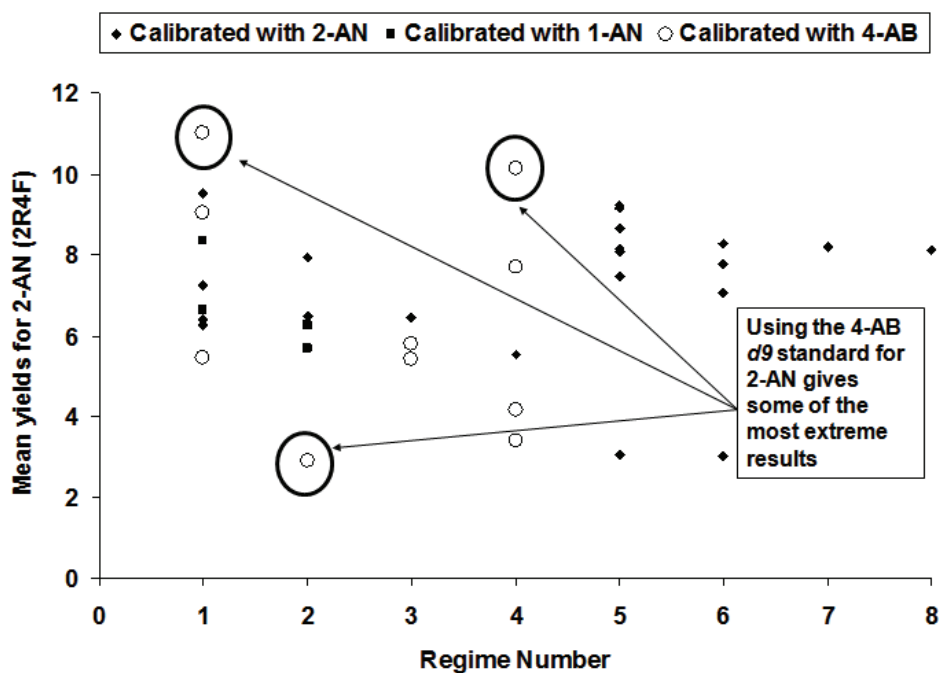
**Appendix 7. Relationship between total particulate matter (TPM) and 1-aminonaphthalene (1-AN) yields across laboratories.** The legends provide the laboratory number.



1-aminonaphthalene yields (ng/cig) from 2R4F cigarettes

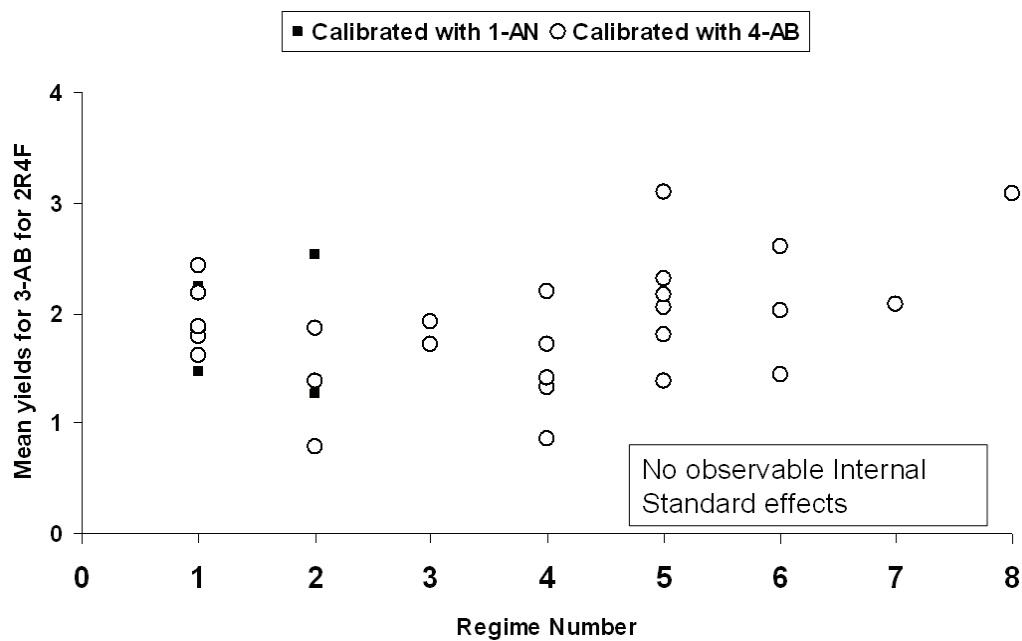


2-aminonaphthalene yields (ng/cig) from 2R4F cigarettes

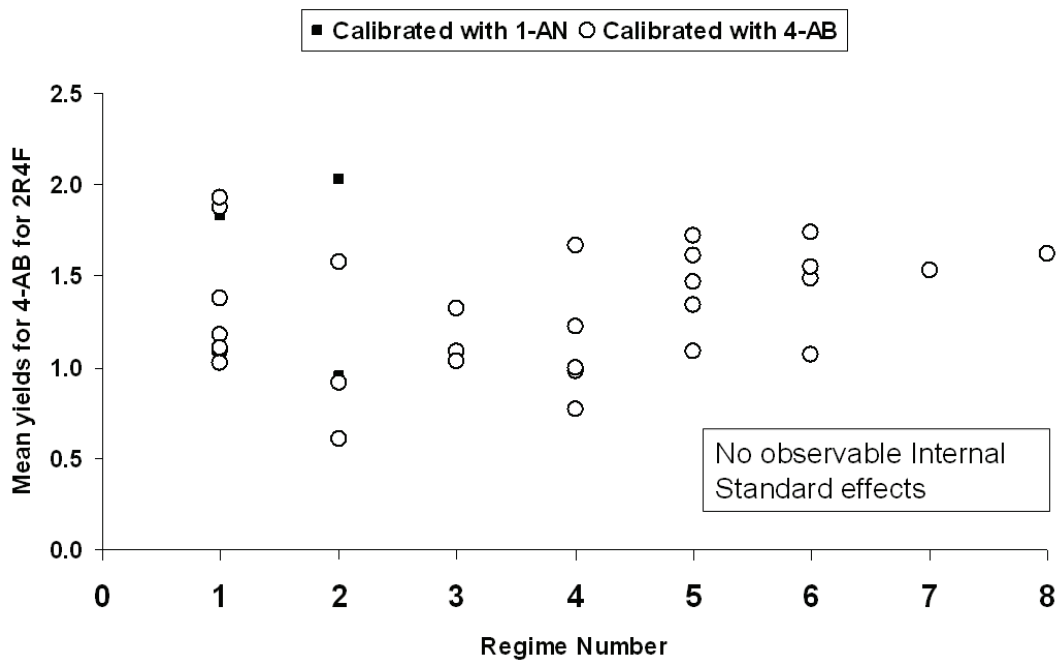


## Appendix 9. Calibrating with different internal standards

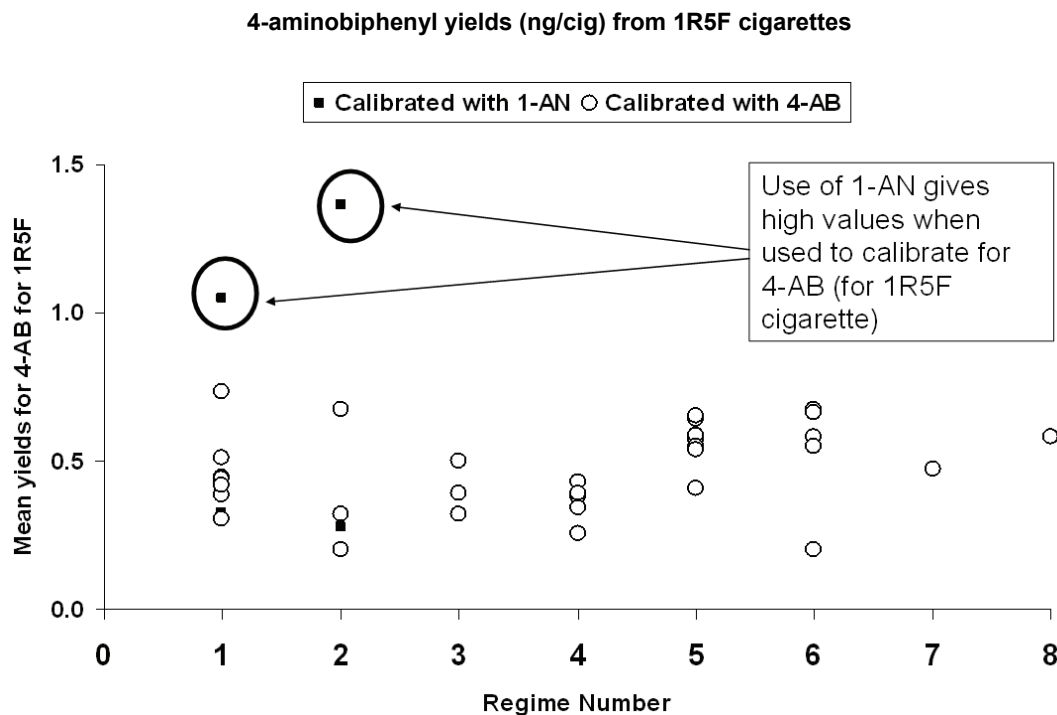
3-aminobiphenyl yields (ng/cig) from 2R4F cigarettes



4-aminobiphenyl yields (ng/cig) from 2R4F cigarettes

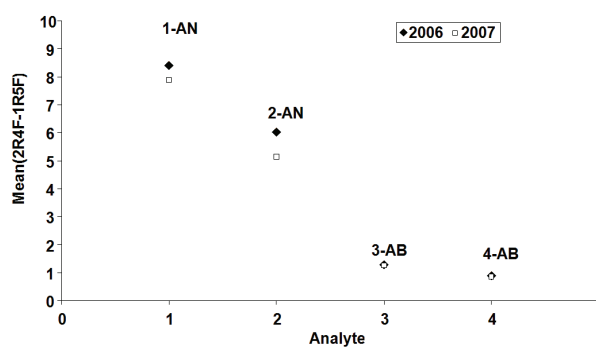


## Appendix 10. Calibrating with different internal standards



**Appendix 11. Comparison of data from 2006 and 2007 CORESTA studies.** Restricted to laboratories numbered 1, 3, 4, 6, 7, 12, 13, 16, 17, 18, 19; 1-AN = 1-aminonaphthalene; 2-AN = 2-aminonaphthalene; 3-AB = 3-aminobiphenyl and 4-AB = 4-aminobiphenyl; % CoV [R (2R4F - 1R5F)] is defined in Appendix 6.

**Mean (2R4F - 1R5F) yields (ng/cig) for each aromatic amine**



**% CoV [R (2R4F - 1R5F)] for each aromatic amine**

