Mainstream Smoke Chemical Analyses for 2R4F Kentucky Reference Cigarette*

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SUMMARY

A new reference cigarette, 2R4F, has been designed to replace the 1R4F Kentucky reference cigarette. This new cigarette has virtually the same blend composition as the 1R4F cigarette. However, the 1R4F cigarette was made in 1983 and the variation in the tobacco from crop year to crop year as well as the difference in the age of the two cigarettes were expected to generate differences in the smoke chemistry. A study done for the quantitation of more than 44 analytes in smoke, including most compounds considered as biologically active, is presented in this report. The analyses were performed by six independent laboratories using a variety of analytical techniques. The smoking was performed using International Standard (ISO) recommendations. The results showed only small differences between the two cigarettes regarding "tar", nicotine and carbon monoxide (CO), as well as for aminonaphthalenes, resorcinol, and some aldehydes. Although the two reference cigarettes were made as close as possible, the concentrations of a significant number of analytes in the smoke differed between 10% to 30%. Specific trace compounds in the blend such as metals and tobacco specific nitrosamines (TSNA), which may influence the smoke composition, were also different between the two cigarettes. The level of lead, in particular, was very different in tobacco between 1983 and 2002. [Beitr. Tabakforsch. Int. 20 (2003) 448–458]

ZUSAMMENFASSUNG

Eine neue Referenzzigarette mit der Bezeichnung 2R4F ist entwickelt worden, um die Kentucky-Referenzzigarette 1R4F zu ersetzen. Diese neue Zigarette hat fast genau die gleiche Mischungszusammensetzung wie die 1R4F Zigarette. Die 1R4F Zigarette wurde jedoch 1983 hergestellt und es war anzunehmen, dass sowohl die Veränderungen im Tabak von Erntejahr zu Erntejahr als auch das unterschied-
liche Alter der beiden Zigaretten zu Unterschieden in der Rauchchemie führen würden. Mehr als 44 Rauchanalyte einschließlich der meisten als biologisch aktiv angesehenen Verbindungen wurden in dieser Studie quantifiziert. Die Analysen wurden von sechs unabhängigen Labors unter Verwendung unterschiedlicher Analysemethoden durchgeführt. Das Abrauchen der Zigaretten erfolgte gemäß ISO (International Organization for Standardization) Richtlinien. Es waren nur kleine Unterschiede bezüglich Kondensat, Nikotin und Kohlenmonoxid (CO) als auch bei den Aminonaphthalinen, Resorcin und einigen Aldehyden zu beobachten. Obwohl die beiden Referenzzigaretten so identisch wie möglich hergestellt wurden, unterschieden sich die Konzentrationen einer signifikanten Anzahl von Rauchanalyten um 10% bis 30%. Es gab auch Unterschiede zwischen den beiden Zigaretten hinsichtlich der spezifischen Spurenbestandteile in der Tabakmischung, wie z.B. den Metallen und tabakspezifischen *N*-Nitrosaminen (TSNA), die Einfluss auf die Zusammensetzung des Rauchs haben könnten. Insbesondere der Bleigehalt unterschied sich im Tabak der Zigaretten von 1983 und 2002 sehr stark. [Beitr. Tabakforsch. Int. 20 (2003) 448–458]

RESUME

Une nouvelle cigarette de référence, 2R4F, a été conçue pour remplacer la cigarette de référence Kentucky 1R4F. Cette nouvelle cigarette a presque la même composition de mélange que la 1R4F. Toutefois, la cigarette 1R4F a été fabriquée en 1983 et il était attendu que les variations du tabac d'une récolte à l'autre ainsi que la différence d'âge entre les deux cigarettes produisent des différences dans la chimie de la fumée. Dans cette étude plus de 44 analytes de la fumée, y compris la plupart des composés considérés comme ayant une activité biologique, ont été dosés. Les analyses ont été réalisées par six laboratoires indépendants faisant appel à plusieurs techniques analytiques. Le fumage a été réalisé selon les conditions normalisées ISO

Table 1. Blend data in % for 1R4F and 2R4F cigarettes (8)

Constituent	1R4F	2R4F
Flue-cured	32.54	32.51
Burley	20.04	19.94
Maryland	1.06	1.24
Oriental	11.09	11.08
Reconstituted (Schweitzer Process)	27.17	27.13
Glycerin	2.80	2.80
Invert sugar	5.30	5.30

Table 2. Cigarette physical data for 1R4F and 2R4F cigarettes (8,9)

Property	1R4F	2R4F
Filter length (mm)	27.2	27.0
Tipping paper length (mm)	32.0	32.0
Cigarette length (mm)	84	84
Cigarette weight (mg)	1085	1060
Open cigarette pressure drop (cm water)	13.8	12.8
Circumference (mm)	24.9	24.8
Filter ventilation (%)	28.4	28.0

Table 3. Some blend chemistry data in % for 2R4F (8)

Compound	Mean	Std. dev.
Total alkaloids	2.31	0.01
Reducing sugar	10.70	0.14
Glycerin	2.44	0.01

(International Organization for Standardization). Les résultats ne révèlent que de légères différences entre les deux cigarettes en ce qui concerne le goudron, la nicotine, et l'oxyde de carbone, ainsi que les aminonaphthalènes, le résorcinol et certains aldéhydes. Bien que la fabrication des deux cigarettes de référence ait été réalisée de manière aussi proche que possible, les concentrations d'un nombre significatif d'analytes de la fumée présentent des variations entre 10% et 30%. Les deux cigarettes se distinguent également par rapport à la teneur en éléments traces spécifiques présents dans le mélange, tels que des métaux et des nitrosamines spécifiques du tabac (TSNA), susceptibles d'influencer la composition de la fumée. En particulier, la teneur en plomb est très différente dans le tabac de 1983 et 2002. [Beitr. Tabakforsch. Int. 20 (2003) 448–458]

INTRODUCTION

The 1R4F cigarette has been used for a long time as a common reference for the analysis of a large number of compounds in mainstream and sidestream smoke. Various publications provide results regarding smoke components for the 1R4F cigarette (1–7). However, the 1R4F cigarette

was made in 1983 and the available stock of cigarettes is significantly reduced. A new cigarette, 2R4F, available from the University of Kentucky, Kentucky Tobacco Research & Development Center (KTRDC), has been designed as close as possible to the 1R4F cigarette with the intention to replace the old reference. Table 1 provides a comparison in the blend composition for the two cigarettes. Table 2 provides a comparison of the cigarette construction. The filler tobacco chemistry of the 1R4F cigarette has been well characterized and presented in various reports (8,9). A few blend chemistry data for the 2R4F cigarette are given in Table 3.

An initial collaborative study on basic smoke characteristics (total particulate matter, nicotine, water, Federal Trade Commission (FTC) "tar", puff count, carbon monoxide (CO) and nitric oxide (NO) for the 2R4F was done with seven participating laboratories. This study has been completed and the data has been released by KTRDC (8). An extended evaluation of smoke for 2R4F cigarettes including most compounds considered as biologically active (10) is reported in this study. These include "tar", nicotine, and CO (TNC), smoke tobacco specific nitrosamines (TSNA), several aromatic amines, carbonyls, volatiles, phenols, mercury, trace metals, hydrogen cyanide, oxides of nitrogen, polycyclic aromatic hydrocarbons (PAHs) including benzo[a]pyrene, pyridine, quinoline, styrene (PQS), and ammonia. Compounds in the tobacco filler that have a direct impact on these smoke components were also analyzed. These include filler TSNA, mercury, and trace metals.

EXPERIMENTAL

A number of analytes were measured and six participating laboratories (Lab A to Lab F) used various analytical techniques for sample collection, sample preparation and measurement of the analytes. The smoking was done following International Standard (ISO) recommendations (11–13). Both Borgwaldt RM20 and linear smoking machines were used, depending on the laboratory or the specific analyte.

A list of the analytes and a summary of each analytical technique for the components in smoke for each laboratory are given in Table 4. The analytes measured in the blend and a summary of the analytical techniques applied for this purpose are given in Table 5. As seen from these tables, some techniques were similar or even identical in different laboratories, while other techniques were different.

Each laboratory measured five replicates for each analyte except for "tar", nicotine, and CO where eight replicates were performed for each sample. The data collected from all laboratories were analyzed as indicated below. The set of data for each analyte formed a matrix $\{x_{i,j}\}$ with the first index "i" indicating the laboratory ($i = 1, \dots p$) and the second index "j" indicating the replicate ($j = 1, \dots n$). The total number of laboratories p was generally equal to six, although for some analytes $p < 6$. The number of replicates $n = 5$, except for "tar", nicotine and CO measurements. Within each laboratory, the average for a lab $\overline{X_i}$ and within the laboratory standard deviation (STD) S_i were calculated. These were obtained using the typical expressions:

Table 4. Summary of analytical techniques used by each laboratory for smoke analysis [a,b]

Analysis	Methods for Lab A	Methods for Lab B	Methods for Lab C	Methods for Lab D	Methods for Lab E	Methods for Lab F
Ammonia	linear, 5 cig/pad + 2 impingers, no cleanup, IC	rotary, 10 cig/acidified pad, no cleanup, IC	NA	rotary, 5 cig/pad + acid in impinger, no cleanup, IC (17)	linear, 5 cig/pad + 1 impinger, cleanup XAD-4, IC	linear, 5 cig in 2 impingers with 0.05 M H_2SO_4, no cleanup, electrochemical
Aromatic amines	linear, 5 cig/pad, liquid/liquid extraction, PFPA derivatization, SIM GC-MS	rotary, 20 cig/pad, SPE cleanup, PFPA derivatization, SIM GC-MS, NCI (14)	NA	rotary, 20 cig/pad, liquid/liquid extraction, PFPA derivatization, SPE cleanup, GC-MS (18)	rotary, 10 cig/pad, no cleanup, HFBA derivatization, GC-MS, NCI	NA
Benzo[a]pyrene	linear, 5 cig/pad, C_{18} SPE cleanup, SIM GC-MS	rotary, 20 cig/pad, SPE cleanup, SIM GC-MS (5)	linear, 5 cig/pad, SPE cleanup, HPLC-fluorescence (15)	linear, 5 cig/pad, SPE cleanup, HPLC-fluorescence (15)	rotary, 20 cig/pad, SPE cleanup, SIM GC-MS	rotary, 20 cig/pad, SPE cleanup, SIM GC-MS
Carbonyls, whole smoke	linear, 5 cig in 2 impingers with DNPH derivatization, HPLC	rotary, 5 cig in 2 impingers with DNPH derivatization, HPLC	linear, 5 cig in 1 impinger with DNPH derivatization, HPLC	linear, 2 cig in 1 impinger with DNPH derivatization, HPLC-UV (19)	linear, 4 cig in 2 impingers, DNPH derivatization, HPLC	linear, 2 cig in 2 impingers, DNPH derivatization, HPLC (19)
Hydrogen cyanide	linear, 3 cig/pad + 1 impinger, no cleanup, colorimetric CFA	rotary, 10 cig/pad, take clearing puffs, GC-MS	linear, 5 cig/pad + impinger, colorimetric CFA	linear, 5 cig/pad + 1 impinger with NaOH solution, analyzed separately, colorimetric CFA (20)	linear, 5 cig in 1 impinger with 1 N NaOH, colorimetric CFA	linear, 5 cig, in 2 impingers with NaOH, colorimetric CFA
Mercury	rotary, 20 cig in 2 impingers, digestion, cold vapor atomic adsorption	rotary, 20 cig, electrostatic + impinger, digestion, cold vapor atomic adsorption	NA	rotary, 20 cig through H_2SO_4/$KMnO_4$ impinger, microwave digestion, cold vapor atomic adsorbtion	NA	measured with other metals
Oxides of nitrogen	linear, 1 cig on line, no cleanup, chemiluminescence	linear, 5 cig, 8 ports, on line, chemiluminescence	NA	1 cig, single port, on line, chemiluminescence	linear, 8 cig, 8 ports, on line, chemiluminescence	NA
Phenols	linear, 5 cig/pad, no cleanup, LC-fluorescence	rotary, 20 cig/pad, derivatization with BSTFA, SIM GC-MS (2)	linear, 5 cig/pad, pad extracted with acetic acid 1% (v/v), HPLC-UV	linear, 5 cig/pad, dilute acetic acid extraction, HPLC-fluorescence (21)	linear, 5 cig/pad, extraction with acetic acid/methanol, HPLC- fluorescence	rotary, 5 cig/pad extraction with TBME, silylation, SIM GC-MS
Pyridine/quinoline	linear, 5 cig in 1 impinger, no cleanup, SIM GC-MS	rotary, 10 cig/acidified pad, solvent extraction, SIM GC-MS (16)	NA	rotary, 20 cig/pad + 1 impinger with cryogenic methanol, no cleanup, GC-MS (7)	same as volatiles, volatiles + pad analyzed together	rotary, 5 cig/pad and XAD-4 sorbent tube, methanol extraction, no cleanup, SIM GC-MS

Table 4. (*cont.*)

Analysis	Methods for Lab A	Methods for Lab B	Methods for Lab C	Methods for Lab D	Methods for Lab E	Methods for Lab F
Styrene	analyzed together with volatiles, no cleanup, GC-MS	analyzed together with volatiles, no cleanup, GC-MS	NA	analyzed together with pyridine and quinoline	same as volatiles, volatiles + pad analyzed together	measured with pyridine and quinoline
TNC	linear, 5 cig, no cleanup, TPM weight, nicotine and water GC, CO non dispersive IR	linear, 5 cig, 8 ports, no cleanup, TPM weight, nicotine and water GC, CO non dispersive IR	linear, 5 cig, 20 ports, no cleanup, TPM weight, nicotine and water GC, CO non dispersive IR	linear, 5 cig, 20 ports, no cleanup, TPM weight, nicotine, water GC (22)	linear, 5 cig, 8 ports, no cleanup, TPM weight, nicotine, water GC	linear 5 cig, 8 ports, no cleanup, TPM weight, nicotine, water GC
Trace metals	rotary, 20 cig, electrostatic, open vessel digestion, ICP-MS	rotary, 20 cig, electrostatic, microwave digestion, ICP AE	NA	rotary, 20 cig, electrostatic + acid impinger, microwave digestion, ICP AE for Ni, Pb, Cd and Cr, GFAAS for As and Se	NA	linear, 8 port, 10 cig, cold traps, adding HNO$_3$ after smoking, ICP-MS
TSNA	linear, 5 cig/pad, no cleanup, LC-MS-MS	rotary, 20 cig/pad, SFE extraction, GC-TEA	linear, 5 cig/pad, SFE extraction, GC-TEA	rotary, 10 cig/pad, solvent extraction + alumina cleanup, GC-TEA (23)	rotary, 20 cig/pad, buffer extraction, CH$_2$Cl$_2$, alumina cleanup, GC-TEA	rotary, 20 cig, direct load on column chromatography with further cleanup, GC-TEA
Volatiles	linear, 5 cig/pad + 1 impinger, no cleanup, GC-MS	rotary, 20 cig in bag, no cleanup, GC-MS (4)	NA	rotary, 10 cig/pad + 2 cryogenic traps with methanol, pad discarded, no cleanup, GC-MS (24)	rotary, 15 cig/pad + 2 cryogenic traps with isooctane/isopropanol 1:1, pad extracted with trap solution, no cleanup, GC-MS	rotary, 5 cig/pad to separate particulate phase, trapping of volatiles in bag, GC-MS

[a] Abbreviations: AE = atomic emission; BSTFA = *N,O*-bis(trimethylsilyl)-trifluoroacetamide; CFA = continuous flow analyzer; DNPH = dinitrophenylhydrazine; GC = gas chromatography; GFAAS = graphite furnace atomic absorption spectroscopy; HFBA = heptafluorobutyric anhydride; HPLC = high performance liquid chromatography; IC = ion chromatography; ICP = inductively coupled plasma; IR = infrared; LC = liquid chromatography; MS = mass spectrometry; NCI = negative chemical ionization; PFPA = pentafluoropropionic anhydride, SFE = supercritical fluid extraction; SIM = single ion monitoring; SPE = solid phase extraction; TBME = *tert*-butyl methyl ether, TEA = thermal energy analyzer; TPM = total particulate matter; UV = ultraviolet.
[b] NA indicates that the technique is not available in the specified laboratory.

Table 5. Summary of analytical techniques used by each laboratory for filler analysis[a,b]

Analysis	Methods for Lab A	Methods for Lab B	Methods for Lab C	Methods for Lab D	Methods for Lab E	Methods for Lab F
Blend mercury	NA	0.3 g tobacco, digestion in open vessel with HNO_3 + H_2O_2, cold vapor atomic adsorption	NA	1 g freeze dry tobacco, microwave digestion (same sample as trace metals), cold vapor atomic adsorption	1 g tobacco, digestion in HNO_3 + H_2O_2, then microwave digestion + H_2O_2, cold vapor atomic adsorption	measured with other metals
Blend trace metals	NA	1 g tobacco, digestion in open vessel with HNO_3 + H_2O_2, ICP-OES	NA	1 g freeze dry tobacco, microwave digestion , ICP for Ni, Pb, Cd and Cr, GFAAS for As and Se	1 g tobacco, digestion in HNO_3 + H_2O_2, then microwave digestion H_2O_2, AA graphite furnace, for As hydride generation with KI, AA determination	0.5 g tobacco, microwave digestion, ICP-MS
Blend TSNA	0.25 g tobacco, buffer extraction, LC-MS-MS	0.5 g tobacco, buffer extraction, CH_2Cl_2 + 0.5 mL NaOH 10% (w/w), GC-TEA	1 g tobacco, extraction with 10 mL CH_2Cl_2 + 0.5 mL NaOH 10% (w/w), GC-TEA	1 g tobacco in ascorbic acid buffer, Chem Elut cleanup, GC-TEA	1g tobacco, buffer extraction, Chem Elut cartridge, CH_2Cl_2 elution, GC-TEA	0.5 g tobacco, ASE, cleanup column chromotography, GC-TEA

[a] Abbreviations: AA = atomic absorption; ASE = accelerated solvent extraction; OES = optical emission spectroscopy.
[b] NA indicates that the technique is not available in the specified laboratory.

$$\overline{X}_i = \frac{1}{n}\sum_{j=1}^{n} x_{i,j} \text{ for } i = 1,2 \ldots p \text{ and}$$

$$S_i = \sqrt{\frac{1}{n-1}\sum_{j=1}^{n}(x_{i,j} - \overline{X}_i)^2} \qquad [1]$$

Among labs average $\overline{X}$ and the standard deviation between each laboratory average value $S_{\overline{X}}$ were calculated using the expressions:

$$\overline{X} = \frac{1}{p}\sum_{i=1}^{p}\overline{X}_i = \frac{1}{pn}\sum_{i=1}^{p}\sum_{j=1}^{n}x_{i,j} \text{ and}$$

$$S_{\overline{X}} = \sqrt{\frac{1}{p-1}\sum_{i=1}^{p}(\overline{X}_i - \overline{X})^2} \qquad [2]$$

The repeatability STD S_r and reproducibility STD S_{-R} were further calculated using the expressions:

$$S_{-r} = \sqrt{\frac{1}{p}\sum_{i=1}^{p}S_i^2} \text{ and } S_{-R} = \sqrt{S_{\overline{X}}^2 + \frac{n-1}{n}S_{-r}^2} \qquad [3]$$

The expression for the coefficients of variation CV_{-r} for repeatability STD and CV_{-R} for reproducibility STD were further obtained as:

$$CV_{-r} = \frac{S_{-r}}{\overline{X}}100\% \text{ and } CV_{-R} = \frac{S_{-R}}{\overline{X}}100\% \qquad [4]$$

The two way-ANOVA (analysis of variance) (25) was used for the statistical comparison.

RESULTS AND DISCUSSION

The results obtained for 1R4F and 2R4F cigarettes for various groups of analytes in smoke or in filler are given in Tables 6 to 12. Table 6 presents the main results for smoke analysis. The results from Table 6 are summarized in Figure 1. This figure shows the absolute values for the % differences between 1R4F and 2R4F smoke data and the corresponding values for CV_{-r} for the measurements on the two cigarettes. The CV_{-r} values describe the precision in the analyses that differentiate the two reference cigarettes. As seen from Figure 1, there were small differences between the two cigarettes regarding "tar", nicotine and CO, as well as for aminonaphthalenes, resorcinol, and some aldehydes. The values for CV_{-r} for about one fourth of the compounds measured in the mainstream smoke of the two cigarettes were larger than % differences in the average values. For the rest of the analyzed compounds, there were larger differences in the smoke of the two cigarettes than the corresponding CV_{-r} values. These results showed that although the two reference cigarettes were made as close as possible, smoke deliveries differed for a number of analytes. Differences as large as 10% to 30% can be seen between some analyte levels in two cigarettes. The statistical significance of those differences was evaluated using the two way-ANOVA analysis. In Table 6 the differences significant at 95% confidence level are identified by an asterisk (*), and the differences significant at 99% confidence level are identified by two asterisks (**).

Table 7 presents the results for some blend analytes. The results from Table 7 are summarized in Figure 2. This figure shows the absolute values for the % differences

Table 6. Results for smoke analysis of 1R4F and 2R4F cigarettes

Method	Analytes	Unit	No. of labs [a]	Average		Rel. diff. (%)[b]	CV_{-r} [c]		CV_{-R} [d]	
				1R4F	2R4F		1R4F (%)	2R4F (%)	1R4F (%)	2R4F (%)
Ammonia	Ammonia	µg/cig	5	12.90	11.02	−15**	6	6	18	11
Aromatic amines	1-Aminonaphthalene	ng/cig	4	15.63	15.06	−4	8	9	28	23
	2-Aminonaphthalene	ng/cig	4	10.40	10.32	−1	10	8	23	22
	3-Aminobiphenyl	ng/cig	4 [e]	3.20	2.97	−7*	7	10	8	9
	4-Aminobiphenyl	ng/cig	4	1.94	1.73	−11**	7	9	19	21
Benzo[a]pyrene	Benzo[a]pyrene	ng/cig	6	5.51	6.96	26**	7	8	23	27
Carbonyls	2-Butanone	µg/cig	6	68.08	62.72	−8**	7	7	30	25
	Acetaldehyde	µg/cig	6	623.88	560.48	−10**	6	5	13	15
	Acetone	µg/cig	6 [e]	293.15	264.74	−10**	6	5	7	5
	Acrolein	µg/cig	6	60.64	58.77	−3	8	7	16	14
	Butyraldehyde	µg/cig	6	33.93	29.58	−13**	9	7	12	9
	Crotonaldehyde	µg/cig	6	15.90	16.18	2	15	11	43	43
	Formaldehyde	µg/cig	6 [e]	22.19	21.61	−3	8	10	9	14
	Propionaldehyde	µg/cig	6	51.54	43.92	−15**	7	5	15	13
Hydrogen cyanide	Hydrogen cyanide	µg/cig	5	128.93	109.20	−15**	5	5	10	9
Volatiles	1,3-Butadiene	µg/cig	5	32.10	29.94	−7**	7	5	26	25
	Acrylonitrile	µg/cig	5 [e]	9.51	8.28	−13**	10	6	16	11
	Benzene	µg/cig	5	44.33	43.39	−2	6	3	19	17
	Isoprene	µg/cig	5	308.08	297.68	−3*	5	4	28	26
	Toluene	µg/cig	5	68.08	64.91	−5*	6	4	34	33
Mercury	Mercury	ng/cig	4	5.43	3.82	−30**	8	9	26	50
Oxides of nitrogen	NO	µg/cig	2	319.88	223.41	−30**	8	6	11	13
	NO_x	µg/cig	2	348.34	268.98	−23**	5	6	6	9
Phenols	Catechol	µg/cig	6 [e]	40.57	37.90	−7**	4	4	7	7
	Hydroquinone	µg/cig	6	42.77	32.40	−24**	5	4	12	14
	m+p-Cresol	µg/cig	6 [e]	7.43	5.84	−21**	7	15	21	25
	o-Cresol	µg/cig	6 [e]	2.62	1.89	−28**	6	7	17	14
	Phenol	µg/cig	6	9.63	7.32	−24**	7	7	38	42
	Resorcinol	µg/cig	5 [e]	0.94	0.91	−3*	3	4	54	54
PQS	Pyridine	µg/cig	5	7.47	7.02	−6	9	10	26	36
	Quinoline	µg/cig	5	0.30	0.23	−22**	7	7	23	19
	Styrene	µg/cig	5	6.13	5.11	−17**	10	9	38	45
Trace metals [f]	Arsenic	ng/cig	3	12.21	10.39	−15	6	18	91	108
	Cadmium	ng/cig	4	55.09	47.82	−13	10	10	30	26
	Chromium	ng/cig	2	57.74	73.01	26				
	Lead	ng/cig	4	42.51	32.95	−23	8	22	51	100
	Nickel	ng/cig	1	6.44	5.12	−20				
	Selenium	ng/cig	3	39.81	34.85	−12	13	16	118	109
TSNA [g]	NAB	ng/cig	6	19.37	16.28	−16**	8	8	15	18
	NAT	ng/cig	6	122.49	119.02	−3	6	7	15	15
	NNK	ng/cig	6 [e]	90.69	115.61	27**	7	6	8	9
	NNN	ng/cig	6	107.09	133.11	24**	4	6	10	12
TNC	TPM	mg/cig	1	12.18	11.3	−8				
	Carbon monoxide	mg/cig	6	12.26	11.96	−2	6	4	6	5
	Nicotine	mg/cig	6	0.80	0.75	−5**	5	5	6	6
	"Tar"	mg/cig	6	9.38	8.91	−5**	4	5	5	6

[a] No. of labs = number of participating laboratories.
[b] The relative difference was defined as (2R4F−1R4F)*100%/1R4F.
[c] CV_r = coefficient of variation of the repeatability standard deviation (26).
[d] CV_R = coefficient of variation of the reproducibility standard deviation (26).
[e] The results of one laboratory were excluded by Dixon Q outliers test for calculating the summary statistics.
[f] Due to inconsistency of the BQLs (below quantification limits) of laboratories, most results were reported as BQL, no statistical comparison was made.
[g] TSNA: N-nitrosoanabasine (NAB), N-nitrosoanatabine (NAT), 4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and N-nitrosonornicotine (NNN).
* Indicates the differences of the two cigarettes were statistically significant at 95% confidence level; ** indicates the differences of the two cigarettes were statistically significant at 99% confidence level. The two way-ANOVA analysis (25) was used for the statistical comparison.
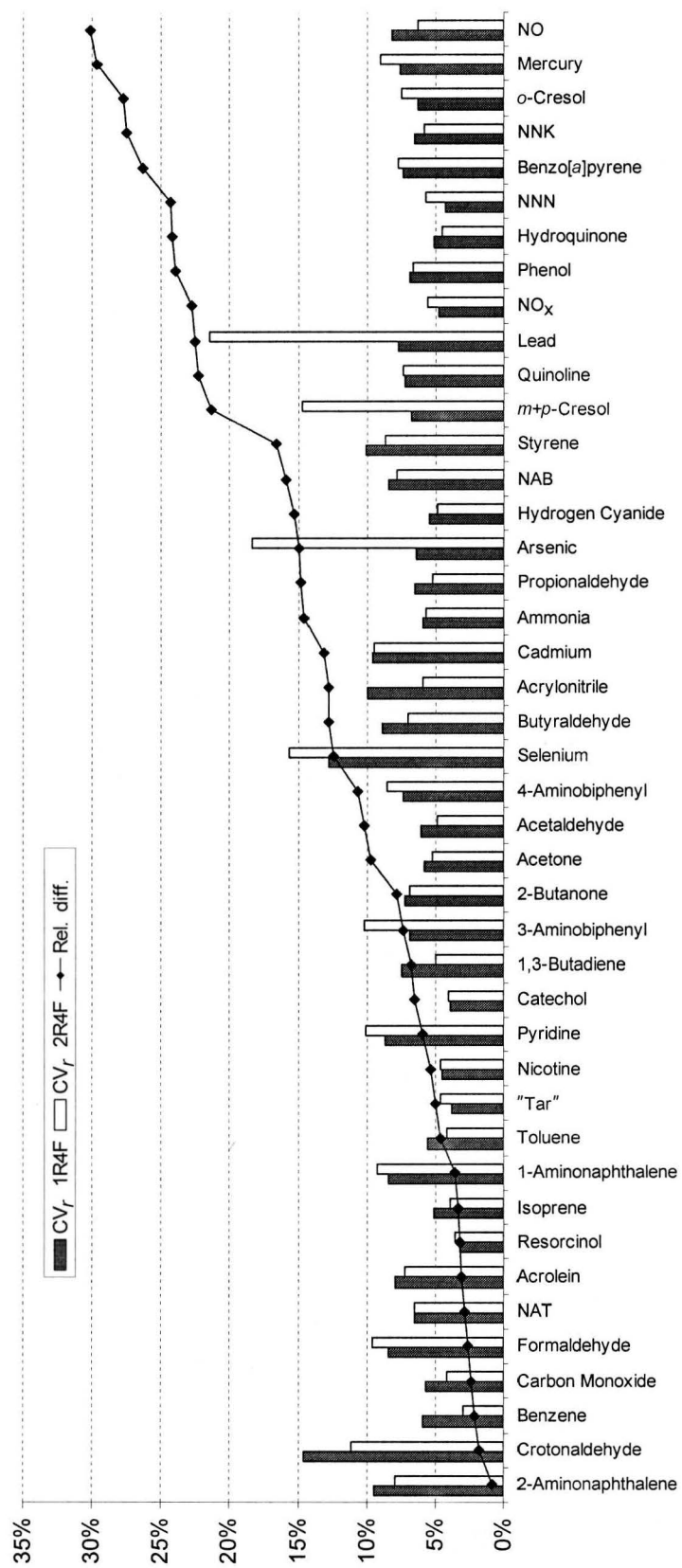
Figure 1. The plot of absolute values for the % differences between 1R4F and 2R4F smoke data and the corresponding values for CV_{-r} for the measurements on the two cigarettes

Table 7. Results for selected blend compounds in 1R4F and 2R4F cigarettes

Method	Analytes	Unit	No. of labs [a]	Average		Rel. diff. (%) [b]	CV_{-r} [c]		CV_{-R} [d]	
				1R4F	2R4F		1R4F (%)	2R4F (%)	1R4F (%)	2R4F (%)
Blend mercury	Blend mercury	µg/g	4	0.05	0.06	23	13	21	55	98
Blend trace metals	Blend arsenic	µg/g	4	0.85	0.77	−10	16	11	69	55
	Blend cadmium	µg/g	4	1.46	1.44	−1	5	3	19	22
	Blend chromium	µg/g	4	1.64	2.18	33**	7	13	49	77
	Blend lead	µg/g	4	1.91	0.72	−62**	8	8	27	34
	Blend nickel	µg/g	4	2.18	2.53	16**	9	10	22	39
	Blend selenium	µg/g	4 [e]	2.98	1.49	−50**	12	27	134	168
Blend TSNA	Blend NAB	ng/g	5	118.14	140.55	19**	12	7	25	16
	Blend NAT	ng/g	5 [e]	1991.75	2473.78	24**	4	2	4	3
	Blend NNK	ng/g	5	1173.02	1791.49	53**	4	4	11	13
	Blend NNN	ng/g	5	2354.44	3539.21	50**	3	3	6	3

[a] No. of labs = number of participating laboratories.
[b] The relative difference was defined as (2R4F−1R4F)*100%/1R4F.
[c] CV_r = coefficient of variation of the repeatability standard deviation (26).
[d] CV_R = coefficient of variation of the reproducibility standard deviation (26).
[e] The results of one laboratory were excluded by Dixon Q outliers test for calculating the summary statistics.
* Indicates the differences of the two cigarettes were statistically significant at 95% confidence level; ** indicates the differences of the two cigarettes were statistically significant at 99% confidence level. The two way-ANOVA analysis (25) was used for the statistical comparison.
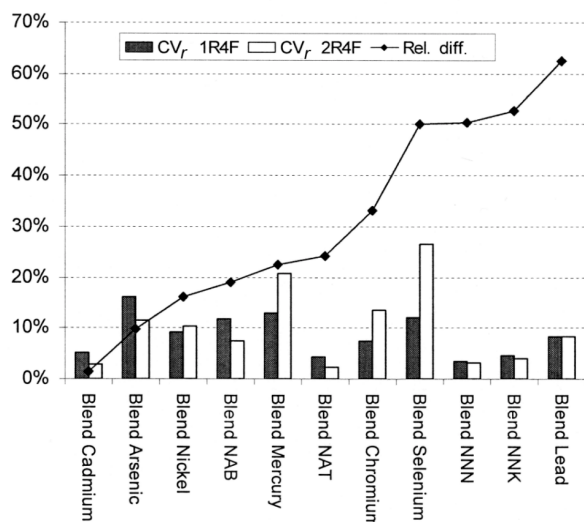
Figure 2. The plot of absolute values for the % differences between 1R4F and 2R4F selected blend compounds, and the corresponding values for CV_{-r} for the measurements on the two cigarettes

between 1R4F and 2R4F selected blend compounds data, and the corresponding values for CV_{-r} for the measurements on the two cigarettes. As seen from Figure 2, specific trace compounds such as metals and TSNA were different in the blend of the two cigarettes. The level of lead, in particular, was very different in tobacco between 1983 and 2002.

Table 6 and Table 7 also contain CV_{-R} data for the analytical measurements used in this study. The CV_{-R} values can be used to characterize the discrepancy between the labs in the measurement of a specific analyte and should not be used in the comparison between the two cigarettes.

Table 8. Results for carbonyl compounds in µg/cig obtained using a GC-MS technique

Analytes	Average (µg/cig)		Std. dev. (µg/cig)	
	1R4F	2R4F	1R4F	2R4F
Acetaldehyde	619.36	583.74	33.99	13.18
Acetone	233.88	261.62	42.45	7.35
Acrolein	47.08	50.34	3.15	1.61
2-Butanone	69.86	73.48	6.23	1.44
Butyraldehyde	12.64	12.64	0.26	0.21
Crotonaldehyde	18.50	20.06	0.91	0.87
Formaldehyde	22.90	23.16	3.39	2.14
Propionaldehyde	46.50	43.96	2.63	0.97

Nevertheless, each CV_{-R} value is a parameter that can be used in judging the accuracy of the result for a specific analyte.

One laboratory analyzed certain carbonyl compounds in mainstream smoke using both high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) techniques. The results from the HPLC method are included in Table 6 together with the data from the other five laboratories. The GC-MS technique was based on the dinitrophenylhydrazine (DNPH) derivatization of carbonyl compounds followed by analysis. The data generated by the GC-MS method are shown in Table 8. The data from Table 8 are slightly lower than the averages shown in Table 6, but in general are in good agreement with those data. However, the results for butyraldehyde were significantly lower by the GC-MS method. The GC-MS method detected 2-methylpropanal at levels about twice those for *n*-butyraldehyde. It is likely that the butyraldehyde reported by the methods using HPLC for separation and detection was not differentiated from the C_4 aldehyde isomer.

Table 9. Results for carbonyl compounds (µg/cig) in the vapor phase of 1R4F and 2R4F cigarettes

	Average (µg/cig)		Std. dev. (µg/cig)	
Analytes	1R4F	2R4F	1R4F	2R4F
Acetaldehyde	484.46	396.78	43.10	71.02
Acetone	209.58	205.52	12.26	8.35
Acrolein	45.96	44.84	3.13	2.52
2-Butanone + n-butyraldehyde	39.52	40.94	4.65	3.62
Crotonaldehyde	6.64	7.1	0.46	0.70
2-Methylpropanal	8.08	8.1	0.34	0.39
Propionaldehyde	32.14	29.6	3.27	2.75

Table 10. Results for other aromatic amines (ng/cig) in the mainstream smoke of 1R4F and 2R4F cigarettes

	Average (ng/cig)		Std. dev. (ng/cig)	
Analytes	1R4F	2R4F	1R4F	2R4F
Aniline	331.40	251.60	16.88	18.09
Benzidine	0.11	0.09	0.02	0.02
2,4-Dimethylaniline	19.06	15.12	1.51	2.16
2,5-Dimethylaniline	15.54	12.46	1.17	1.91
2,6-Dimethylaniline	6.28	3.93	0.63	0.53
2-Ethylaniline	8.75	6.84	0.67	0.89
3,4-Dimethylaniline	13.32	8.23	0.59	1.17
3,5-Dimethylaniline	9.05	6.77	0.64	1.07
3-Ethylaniline	10.09	7.96	0.74	1.25
4-Ethylaniline	8.75	6.48	0.59	1.02
m-Toluidine	55.22	46.26	0.57	4.71
o-Toluidine	49.76	42.42	0.59	2.72
p-Toluidine	41.56	29.68	1.57	3.23
Tolidine	0.03	ND [a]	0.02	—

[a] ND = not detected.

Table 11. Results for other polycyclic aromatic hydrocarbons (ng/cig) in the mainstream smoke of 1R4F and 2R4F cigarettes

	Average (ng/cig)		Std. dev. (ng/cig)	
Analytes	1R4F	2R4F	1R4F	2R4F
Anthracene	39.16	45.82	2.14	4.17
Benzanthracene	10.37	14.48	0.89	0.89
Benzo[e]pyrene	3.60	4.67	0.17	0.39
Benzofluoranthene	8.02	10.24	0.64	0.70
Benzofluorene	29.38	34.20	2.05	2.90
Benzoperylene	1.16	1.52	0.06	0.15
Chrysene	15.66	20.50	0.57	2.12
Dibenanthrancene	0.38	0.46	0.05	0.09
Fluoranthene	46.04	56.20	1.54	6.28
Fluorene	116.40	119.80	9.07	9.26
Naphthalene	339.60	271.60	20.77	18.74
Perylene	0.67	0.88	0.09	0.11
Phenanthrene	94.14	125.20	4.86	11.01
Pyrene	29.64	39.20	1.56	239

Table 12. Results for nicotine, ammonia and some minor alkaloids (mg/g tobacco) for 1R4F and 2R4F cigarettes

	Average (ng/cig)		CV $_r$	
Analytes	1R4F	2R4F	1R4F	2R4F
Nicotine	17.83	19.66	1.30	1.16
Ammonia	805.14	1429.46	1.71	3.56
Anabasine	0.14	0.14	3.30	2.82
Anatabine	0.83	0.90	0.91	1.38
Myosmine	0.08	0.10	5.65	5.65
Nornicotine	0.83	1.16	3.61	3.27

The carbonyl compounds in the vapor phase of the two reference cigarettes were also measured in only one laboratory. Twenty cigarettes were smoked on a rotary machine and the vapor phase collected in a plastic smoke bag (4). The vapor phase was then immediately injected in a GC-MS system equipped with a PoraPlot Q type column (from Chrompack/Varian). The data are shown in Table 9. As expected, the levels of the carbonyl compounds in vapor phase were lower than those in total mainstream smoke. The compound 2-methylpropanal was also identified in the vapor phase of mainstream smoke.

In addition to the commonly analyzed aminonaphthalenes and aminobiphenyls, a number of other aromatic amines present in smoke were measured by one of the participating laboratories. The results for these amines are given in Table 10. A number of other PAHs present in smoke, besides benzo[a]pyrene, were also measured by one of the participating laboratories. The results for these PAHs are given in Table 11. The results from Tables 10 and 11 show differences between the two reference cigarettes in the same range as for other analytes (up to about 30%). The aromatic amines are typically lower in the 2R4F cigarette while the PAHs are lower in 1R4F (except for naphthalene).

Two laboratories also determined the level of nicotine, ammonia, and of several minor alkaloids in the tobacco blend. The results are shown in Table 12. Although the nicotine levels in the 1R4F blend were lower than in 2R4F the level of this compound in smoke was slightly higher in 1R4F cigarettes. The same observation can be made for ammonia and smoke ammonia in the two cigarettes.

CONCLUSIONS

A number of analytes in smoke including most compounds considered as biologically active were analyzed in 1R4F and 2R4F Kentucky reference cigarettes. A few compounds in the tobacco section that may be transferred in smoke were also analyzed. The study was done in six independent laboratories to generate generally accepted average values for the specific analytes measured in this study. The cigarettes were smoked following ISO recommendations. The results showed that regarding "tar", nicotine and CO, there are only small differences between the two cigarettes. The same situation was true for a few compounds such as aminonaphthalenes, resorcinol, and some aldehydes. For the rest of the analyzed compounds there were larger differences in the smoke of the two cigarettes. Although the two reference cigarettes were made as close as possible, a significant number of analytes differed 10% to 30% between the two cigarettes.

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