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The effect of tobacco ingredients on smoke chemistry. Part II: Casing ingredients

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Abstract

This is the second part of a study in which the effects of adding a range of ingredients to tobacco on the chemistry of cigarette mainstream smoke are assessed. The examination of smoke chemistry has concentrated on those constituents in smoke that regulatory authorities in the USA and Canada believe to be relevant to smoking-related diseases. In this part of the study the effects of 29 casing ingredients and three humectants have been assessed at the maximum levels typically used on cigarettes by British American Tobacco. This brings the total number of ingredients assessed in Parts I and II of this study to 482. The casing ingredients were added at levels of up to 68 mg on the cigarettes. Their effects on smoke constituents were generally larger than the effects of flavouring ingredients, which were added at parts per million levels. Many of the casing ingredient mixtures either had no statistically significant effect on the level of the analytes investigated in smoke relative to a control cigarette, or they produced decreases of up to 44% in some cases. Those analytes that were increased in smoke are highlighted in this paper. The largest increases were for formaldehyde levels, up to 26 μg (73%) in one case, observed from casing mixtures containing sugar. This is most likely due to the generation of formaldehyde by pyrolysis of sugars. Occasional small increases were also observed for other analytes. However, the statistical significance of many of these increases was not present when the long-term variability of the analytical method was taken into account. The significance and possible reasons for the increases are discussed.

Keywords: Casing; Chemical analysis; Cigarette; Constituent; Ingredient; Smoke; Tobacco

1. Introduction

This is the second part of a study in which the effects of adding a range of ingredients to tobacco on the chemistry of cigarette mainstream smoke are assessed. Part I of the study addressed the effects of flavouring ingredients, a few casings and additives on those smoke constituents that are believed by regulatory authorities in the USA and Canada to be relevant to smoking-related diseases, colloquially called ''Hoffmann analytes'' (Baker et al., in press). In this second part of the study, the effects of a range of casing ingredients on smoke chemistry are considered.

2. Materials and methods

The base tobacco was a typical US blend containing lamina and reconstituted tobacco sheet (Table 1). Seven mixtures of casing ingredients were prepared in propylene glycol and water and added to this base tobacco blend, using normal manufacturing techniques at application levels of 15 or 20% (dry-weight basis) in the final cased tobacco blend (Table 2). An additional mixture used in cigarette code C6 consisted of the propylene glycol and water only. The resultant cased tobaccos were made into a series of cigarettes with identical design features, coded C1 (control, containing the base tobacco) to C9 (Table 3). This Experimental Cigarette Series C follows on from series A and B used in Part I of the study (Baker et al., in press). The design features of the experimental cigarettes were deliberately chosen so as to produce cigarettes that gave relatively high ''tar'' yields, approximately 13 mg under the standard ISO

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Table 1
Composition of base tobacco blend in Experimental Cigarette Series C

Constituent	Level on blend[a] (%)	CAS Number[b]
Lamina	82.1	
Reconstituted sheet	17.9	
Cellulose powder	1.25	65996-61-4 9004-34-6
Cocoa powder	1.07	84649-99-0
Diammonium hydrogen phosphate	0.90	7783-28-0
Licorice powder	1.07	68916-91-6 84775-66-6
Propylene glycol	0.18	57-55-6
Tobacco	13.4	

[a] Dry-weight basis.
[b] Chemical Abstract Service Registry Number.

machine smoking conditions (ISO 3308, 2000). This was so that any effects of the ingredients would be maximised and readily observed. With low "tar" cigarettes the levels of some of the "Hoffmann analytes" in smoke would be near their detection limits.

The combinations of casing ingredients used in these experimental cigarettes were for experimental convenience. These combinations are not necessarily used in any commercial cigarette. The percentages and levels of individual ingredients on the tobacco reflect maximum levels typically used on cigarettes sold by British American Tobacco.

The cigarettes were manufactured in the R&D Centre of British American Tobacco in the UK and sent to the R&D Division of the British American Tobacco company in Brazil (Souza Cruz), for analysis of smoke constituents and some tobacco properties. The cigarettes were machine smoked under the standard ISO machine smoking regime of one 35 ml puff of 2 s duration taken every minute to a butt length equal to the filter length + 8 mm, under ambient conditions of 22 °C and 60% relative humidity (ISO 3308, 2000). A series of smoking runs was undertaken and groups of analytes were determined following each smoking run. The groups of analytes measured together are as shown in the subdivisions of results in Tables 4–9. The analytical methodology used was identical to that described in Part I of this study (Baker et al., in press).

3. Results and discussion

A total of 38 ingredients has been used in Part II of the study, including propylene glycol and water (Table 2). Of these ingredients, six were also included in the numbers of ingredients cited in Part I of the study (Baker et al., in press). Consequently 32 ingredients were tested for the first time in this part of the study, 29 casing ingredients and three humectants.

The results of the mainstream smoke analyses, together with some tobacco blend analyses, are given in Tables 4–9 for Experimental Cigarette Series C. In these tables it is indicated where there is a statistically significant difference at the 95% confidence level between the means of the control cigarette, C1, and the test cigarettes containing flavour mixtures, C2 to C9, determined using a Student's *t*-test with unknown and unequal variances. Since it was not known in which direction the differences might lie, a two-tailed test was used. As discussed in Part I of this paper, for those pairs of test and control cigarettes where there was a statistically significant difference, the significance was calculated again, taking into account the long-term variability of the analyte methodology. This was done using the pro rata standard deviations obtained with the University of Kentucky 1R4F reference cigarette (Baker et al., in press). Significant differences obtained using this wider analyte level variation are also indicated in the tables.

Several ingredients were added to each of the test cigarettes C2–C9. Any change in the yields of smoke constituents relative to the control cigarette (C1) may be the result of an individual ingredient or of several different ingredients.

The total smoking material weight (tobacco + casing ingredients) was kept constant in the cigarettes C1–C9 to within 4%. The test cigarettes were loaded with 51–68 mg of casing ingredients, excluding propylene glycol and water (Table 2). Consequently, the amount of tobacco in the test cigarettes (C2–C9) was lower than that in the control (C1) by up to 68 mg. (About 55–65% of the 6 mg of propylene glycol will be lost from tobacco during manufacture and storage (Baker et al., in press). Final water levels in all cigarettes will be similar as they are all conditioned at 60% relative humidity).

Cigarette code C6 had only propylene glycol and water added to the tobacco (Table 2). The conditioned cigarette weight was 4% lower than the control (C1), Table 4. With this cigarette, almost all of the smoke analyte levels were either not statistically different to the control (Tables 4–9) or had their levels reduced by up to 28% (phenol, Table 7). The level of propionaldehyde in smoke in C6 was significantly increased by 12% relative to the control (Table 7). This was out of line with the other smoke carbonyl constituents for this cigarette, which were either the same as the control, or reduced by 15% (formaldehyde). The increased level for propionaldehyde was not significant when the long-term variability of the analytical methodology was taken into account. The levels of three of the metals in the tobacco blend were significantly increased relative to the control, nickel by 74% (Table 9). It is difficult to rationalise these increases in metal levels and, in fact, the metal

Table 2
Individual ingredients used in experimental mixtures for Experimental Cigarette Series C

Cigarette code	Ingredient	Function of ingredient	CAS number	Level added to tobacco blend[a]	
				%	mg
C2	Cocoa powder	Flavour	84649-99-0	3.77	22.7
	Corn syrup, high fructose	Flavour	8029-43-4	6.20	37.3
	Propylene glycol	Humectant	57-55-6	1.00	6.00
	Water	Process aid	7732-18-5	4.03	24.3
	Total ingredients			15.0	90
C3	Acetic acid	Flavour	64-19-7	0.90	5.42
	Sugar, white	Flavour	57-50-1	10.5	63.2
	Propylene glycol	Humectant	57-55-6	1.00	6.00
	Water	Process aid	7732-18-5	7.60	45.8
	Total ingredients			20.0	120
C4	Glycerol	Flavour	56-81-5	7.00	42.1
	Malic acid	Flavour	6915-15-7	0.65	3.91
	Prune extract	Flavour	90082-87-4	1.74	10.5
	Propylene glycol	Humectant	57-55-6	1.00	6.00
	Water	Process aid	7732-18-5	4.61	27.8
	Total ingredients			15.0	90
C5	Apricot extract	Flavour	68650-44-2	0.06	0.36
	Citric acid	Flavour	77-92-9	1.14	6.86
	Honey	Flavour	8028-66-8	4.54	27.3
	Orris root extract	Flavour	8002-73-1	0.01	0.06
	Pectin	Flavour	9000-69-5	0.03	0.18
	Plum extract	Flavour	90082-87-4	0.02	0.12
	Sorbitol	Humectant	50-70-4	3.53	21.3
	Propylene glycol	Humectant	57-55-6	1.00	6.00
	Water	Process aid	7732-18-5	4.67	28.1
	Total ingredients			15.0	90
C6	Propylene glycol	Humectant	57-55-6	8.33	50.1
	Water	Process aid	7732-18-5	6.67	40.2
	Total ingredients			15.0	90
C7	Chocolate	Flavour	N/A	0.28	1.69
	Cocoa extract	Flavour	84649-99-3	2.00	12.0
	Corn syrup	Flavour	68131-37-3	6.20	37.3
	Propylene glycol	Humectant	57-55-6	1.00	6.00
	Water	Process aid	7732-18-5	5.52	33.2
	Total ingredients			15.0	90
C8	Alfalfa extract	Flavour	84082-36-0	0.01	0.06
	Carob bean extract	Flavour	84961-45-5	0.50	3.01
	Chicory extract	Flavour	68650-43-1	0.01	0.06
	Coffee extract	Flavour	93348-12-0	0.27	1.63
	Fenugreek extract	Flavour	84625-40-1	0.02	0.12
	Fig extract	Flavour	68916-52-9	1.17	7.04
	Kola nut extract	Flavour	68916-19-8	0.01	0.06
	Lactic acid	Flavour	598-82-3	1.24	7.46
	Maple syrup	Flavour	91770-22-8	0.01	0.06
	Mate extract	Flavour	68916-96-1	0.05	0.30
	Molasses, sugar cane	Flavour	68476-78-8	5.35	32.2
	Oak chip extract	Flavour	68917-11-3	0.01	0.06
	Raisin extract	Flavour	68915-56-6	1.14	6.86
	Rye extract	Flavour	91770-60-4	0.01	0.06
	Tea extract, absolute	Flavour	68916-73-4	0.03	0.18
	Valerian root extract	Flavour	8057-49-6	0.06	0.36
	Propylene glycol	Humectant	57-55-6	1.00	6.00

(continued on next page)

Table 2 (*continued*)

Cigarette code	Ingredient	Function of ingredient	CAS number	Level added to tobacco blend[a]	
				%	mg
C8 (continued)	Water	Process aid	7732-18-5	4.11	24.7
	Total ingredients			15.0	90
C9	Licorice extract, powder	Flavour	68916-91-6	2.00	12.0
	Sugar, invert	Flavour	8013-17-0	7.00	42.1
	l-Tartaric acid	Flavour	87-69-4	0.75	4.51
	Propylene glycol	Humectant	57-55-6	1.00	6.00
	Water	Process aid	7732-18-5	4.25	25.6
	Total ingredients			15.0	90

N/A, Not available.

[a] In addition, some ingredients also present from the base tobacco blend (see Table 1). % on dry-weight basis. Weight added to cigarette calculated using 704 mg as weight of moist, cased tobacco and tobacco moisture content of 14.5%.

Table 3
Design features of experimental cigarettes C1–C9

Total length (mm)	83.5
Tobacco rod length (mm)	62.5
Filter length (mm)	21.0
Filter tipping length (mm)	25.0
Circumference	24.70
Total cased-tobacco filler weight (mg)	704
Total cigarette weight (mg)	906
Paper permeability (CU[a])	80
Paper additive	Ammonium phosphate (0.45%)
Filter ventilation (%)	0
Tobacco	US lamina/recon. + various casings (see Table 2)

[a] CORESTA units of permeability, cm min^{-1} kPa^{-1}.

levels in smoke from cigarette C6 were not significantly different to the control, C1, albeit some were below their limits of detection or quantitation.

Relative to the control cigarette (C1), all the cigarettes containing casing ingredients had their "tobacco pH" significantly reduced by up to 0.39 pH units (Table 4). Most of these decreases remained significant even when the long-term variability of the method was taken into account. In parallel with this, the "smoke pH" from the cigarettes containing the casing ingredients was also significantly reduced relative to the control, by up to 0.35 pH units.

The amount of smoke total particulate matter (TPM) from cigarette C4 was increased by 1.9 mg (10%) relative to the control (Table 4). This was most probably due to the presence of 42 mg glycerol in the ingredient mixture of this cigarette (Table 2). Glycerol on tobacco transfers largely intact to smoke (Baker and Bishop, in press) and contributes completely to the total particulate matter of smoke.

The presence of the casing ingredients generally decreased the level of nicotine in tobacco, by up to 14% relative to the control (Table 6). Some of the other tobacco alkaloids were also reduced, myosmine by 19% in cigarette C3. Some of these decreases remained significant when the long-term variability of the analytical method was taken into account. The percentage decreases were generally similar to the 'dilution' effect of the presence of the casing ingredients, equal to about 6–13%, assuming 60% of the propylene glycol and about half of the water added will be lost from the tobacco during manufacture and storage (Table 2). The decrease in myosmine for C3 (19%) was greater than the dilution effect (13%). The presence of four of the casing ingredient mixtures also significantly reduced the level of nicotine in the smoke relative to the control, by up to 13% (Table 4).

The presence of the casing ingredients either had no significant effect on CO levels in smoke, or they produced decreases of up to 7% relative to the control (Table 4).

The presence of the casing ingredient mixtures generally had no significant effect on the level of tobacco specific nitrosamines in the tobacco, with the exception that four of the test cigarettes had their levels of tobacco NAB reduced by up to 24% (Table 5). This was greater than the 'dilution' effect of the ingredients. The levels of NNN in smoke were reduced in all the test cigarettes relative to the control, by up to 30% (Table 5). With one exception (cigarette C5), the reductions all remained significant when the long-term variability of the analytical methodology was taken into account. Reductions of up to 27% were also observed for smoke NAT, NAB and NNK in some of the test cigarettes. The total levels of tobacco specific nitrosamines were significantly reduced by up to 27% in the smoke from all the test cigarettes. For three of the test cigarettes these reductions remained significant when the long-term variability of the analytical methodology was taken into account.

The presence of the ingredients had no significant effect on the levels of ammonium ion in the tobacco blend, and generally decreased the level of nitrate by up

Table 4
Experimental Cigarette Series C: standard smoke yields, pH and filter filtration efficiencies

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Cigarette weight (mg)	894	902	884	897	898	859[a,b]	874[a,b]	889	897
Stand. Dev. ($n=4$)	4.03	7.19	6.99	9.81	3.32	9.91	9.29	8.58	4.43
Puff count	7.6	7.6	7.4	7.7	7.7	7.1[a,b]	7.3[a,b]	7.7	7.6
Stand. Dev. ($n=8$)	0.24	0.25	0.14	0.18	0.18	0.14	0.22	0.22	0.38
TPM (mg/cig)	18.6	17.6[a]	18.5	20.5[a,b]	18.7	17.6	18.4	18.1	17.8
Stand. Dev. ($n=8$)	0.984	0.477	0.661	0.881	0.806	0.980	0.947	1.05	0.950
NFDPM[c] (mg/cig)	13.3	12.9	13.3	14.3[a,b]	13.5	12.6[a]	13.1	13.1	13.3
Stand. Dev. ($n=8$)	0.511	0.416	0.339	0.561	0.507	0.703	0.525	0.511	0.423
Nicotine (mg/cig)	1.02	0.98	0.94[a,b]	0.92[a,b]	0.99	0.89[a,b]	0.94[a,b]	1.00	0.98
Stand. Dev. ($n=8$)	0.051	0.035	0.032	0.063	0.051	0.045	0.031	0.027	0.024
CO (mg/cig)	15.7	15.3	14.9[a]	15.6	15.5	14.8[a]	14.9[a]	14.6[a,b]	14.7[a]
Stand. Dev. ($n=8$)	0.559	0.873	0.678	0.515	0.318	0.686	0.835	1.02	0.607
'Smoke pH'	6.27	6.13[a]	5.94[a,b]	5.96[a,b]	6.04[a]	6.04[a]	5.97[a,b]	5.92[a,b]	6.04[a]
Stand. Dev. ($n=5$)	0.0365	0.0303	0.0800	0.0316	0.0371	0.0583	0.0344	0.0305	0.0390
'Tobacco pH'	5.71	5.63[a,b]	5.50[a,b]	5.42[a,b]	5.32[a,b]	5.70[a]	5.65[a,b]	5.38[a,b]	5.47[a,b]
Stand. Dev. ($n=5$)	0.0045	0.0055	0.0045	0.0045	0.0045	0.0045	0.0045	0.0045	0.0045
Filter Filtr. Efficiency (%)									
TPM	42.1	42.7	41.8	40.6	44.8[a,b]	42.9	40.8	43.3	42.2
Stand. Dev. ($n=5$)	2.24	1.61	1.13	2.22	1.07	2.12	0.50	1.84	2.70
Nicotine	41.7	41.7	40.9	41.4	41.0	43.8[a]	40.5[a,b]	39.8[a,b]	40.4[a,b]
Stand. Dev. ($n=5$)	0.57	0.99	0.83	2.76	0.85	1.67	0.33	0.59	0.45

[a] Significantly different to C1 (control) value at 95% confidence level—this data set.
[b] Also significantly different to C1 (control) value at 95% confidence level when long-term analytical method variability is included.
[c] Nicotine-free dry particulate matter (or "tar").

Table 5
Experimental Cigarette Series C: tobacco specific nitrosamines[a]—blend and smoke yields

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Blend (ng/g) (wet basis)									
NNN	1757	1794	1702	1716	1840	1821	1879[b]	1747	1684
Stand. Dev. ($n=5$)	40.4	82.1	105	82.0	76.0	61.4	108	78.9	65.7
NAT	1146	1184	1096	1114	1171	1190	1218	1109	1097
Stand. Dev. ($n=5$)	42.6	46.0	52.6	60.0	52.4	69.8	83.9	63.3	69.6
NAB	74.5	57.7[b]	56.9[b]	64.6[b]	64.5[b]	67.3	77.3	65.3	75.6
Stand. Dev. ($n=5$)	3.94	2.99	8.33	5.38	5.09	16.8	5.07	9.49	7.92
NNK	475	493	459	458	470	482	473	464	446
Stand. Dev. ($n=5$)	36.5	28.5	23.6	22.3	39.6	46.9	31.0	43.6	33.7
TOTAL	3453	3528	3313	3352	3545	3561	3650	3386	3302
Stand. Dev. ($n=5$)	117.6	138.7	154.5	143.8	130.9	179.4	215.3	165.7	158.5
Smoke (ng/cig)									
NNN	161	133[b,c]	112[b,c]	116[b,c]	147[b]	134[b,c]	136[b,c]	131[b,c]	133[b,c]
Stand. Dev. ($n=5$)	4.42	2.78	1.82	1.77	2.85	4.54	4.47	6.75	6.67
NAT	103	103	77.1[b,c]	95.3	105	107	103	100	97.5
Stand. Dev. ($n=5$)	6.23	4.49	6.77	4.59	1.66	3.14	5.05	6.22	3.48
NAB	23.7	20.8	17.3[b,c]	19.6	20.6	23.3	23.3	19.3[b]	19.3[b]
Stand. Dev. ($n=5$)	3.00	3.96	1.64	3.52	1.82	2.87	2.06	2.93	2.44
NNK	68.3	68.4	53.6[b,c]	55.9[b,c]	62.8	63.8	61.6	60.8[b,c]	70.1
Stand. Dev. ($n=5$)	4.15	1.90	4.29	1.64	4.40	3.06	5.76	2.48	8.14
TOTAL	356	325[b]	260[b,c]	287[b,c]	335[b]	328[b]	324[b]	311[b,c]	320[b]
Stand. Dev. ($n=5$)	15.8	9.54	9.65	8.34	8.85	8.12	15.0	14.5	13.3

[a] NNN, *N*′-nitrosonornicotine; NAT, *N*′-nitrosoanatabine; NAB, *N*′-nitrosoanabasine; and NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.
[b] Significantly different to C1 (control) value at 95% confidence level—this data set.
[c] Also significantly different to C1 (control) value at 95% confidence level when long-term analytical method variability is included.

Table 6
Experimental Cigarette Series C: nitrogenous substances—blend and smoke yields

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Blend (wet basis)									
NH_4^+ (μg/g)	2100	2080	1950	2010	2160	2095	2130	2110	2100
Stand. Dev. ($n=5$)	170	106	148	103	71.8	76.8	198	129	122
NO_3^- (%)	0.80	0.78[a]	0.72[a,b]	0.74[a,b]	0.77[a]	0.75[a,b]	0.75[a,b]	0.74[a,b]	0.74[a,b]
Stand. Dev. ($n=5$)	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01
Blend alkaloids (%, wet basis)									
Nicotine	1.74	1.58[a,b]	1.49[a,b]	1.60[a,b]	1.62[a]	1.62[a]	1.64[a]	1.62[a]	1.64[a]
Stand. Dev. ($n=5$)	0.0223	0.0186	0.0095	0.0185	0.0095	0.0104	0.0097	0.0175	0.0105
Nornicotine	0.121	0.115[a]	0.106[a]	0.119	0.109[a]	0.109[a]	0.106[a]	0.108[a]	0.111[a]
Stand. Dev. ($n=5$)	0.0027	0.0020	0.0005	0.0011	0.0010	0.0013	0.0033	0.0010	0.0018
Anabasine	0.0130	0.0113[a,b]	0.0112[a,b]	0.0123	0.0132	0.0132	0.0129	0.0134	0.0131
Stand. Dev. ($n=5$)	0.0010	0.0005	0.0002	0.0003	0.0005	0.0005	0.0002	0.0004	0.0004
Anatabine	0.0940	0.0872[a]	0.0836[a]	0.0927	0.0954[a]	0.0932	0.0865[a]	0.0897[a]	0.0891[a]
Stand. Dev. ($n=5$)	0.0010	0.0012	0.0004	0.0008	0.0007	0.0021	0.0027	0.0008	0.0013
Myosmine	0.00910	0.00752[a]	0.00735[a,b]	0.00782[a]	0.00904	0.00902	0.00873	0.00925	0.00944
Stand. Dev. ($n=5$)	0.00087	0.00019	0.00031	0.00034	0.00051	0.00060	0.00025	0.00041	0.00073
Smoke (μg/cig)									
NH_3	24.0	23.2	24.2	31.9[a,b]	21.8	24.0	18.0[a,b]	26.5[a]	17.8[a,b]
Stand. Dev. ($n=5$)	1.33	1.58	0.60	1.71	2.22	2.84	1.28	1.13	1.83
NO_X	266	273	237[a,b]	246[a]	269	261	255[a]	252	261
Stand. Dev. ($n=5$)	4.82	13.8	10.2	9.71	6.16	5.14	2.87	13.2	7.55
HCN	238	241	224	236	251	216[a]	234	252	274[a,b]
Stand. Dev. ($n=5$)	13.4	7.49	10.9	8.87	14.1	6.39	4.57	10.3	13.7

[a] Significantly different to C1 (control) value at 95% confidence level—this data set.
[b] Also significantly different to C1 (control) value at 95% confidence level when long-term analytical method variability is included.

to 10% (Table 6). The latter was probably a 'dilution' effect of the ingredients.

With one exception, the presence of the ingredients also had no significant effect, or decreased the levels of HCN and NO_x in smoke by up to 11% (Table 6). The exception was test cigarette C9, whose smoke level of HCN was increased by 36 μg (15%) relative to the control cigarette C1, this increase remaining significant when the long-term variability of the method was taken into account. The casing on C9 contained licorice extract powder, invert sugar, *l*-tartaric acid, propylene glycol and water (Table 2). Comparison of this increase in HCN with HCN results for other ingredient mixtures helps to narrow down identification of the ingredient that may be responsible for this increase in HCN. Sample C6 had added propylene glycol and water (Table 2) and a significantly lower smoke HCN yield than the control (Table 6), so they were not responsible. The same level of licorice extract powder was added to cigarette B4 in Part I of this study (Baker et al., in press) and that cigarette has the same HCN smoke yield as its control, thus eliminating licorice. This leaves invert sugar or *l*-tartaric acid. Cigarette C3 had 10.5% white sugar as an ingredient (Table 2) and no increased smoke HCN (Table 6). However, cigarette B2 had 6.2% brown sugar and a smoke HCN yield increased by 24% relative to its control (Baker et al., in press). Consequently, with test cigarette C9 in the present study, some reaction involving invert sugar may have been responsible for the increased HCN level.

The levels of NH_3 in smoke from cigarettes C7 and C9 were reduced by 6.0–6.2 μg (25%) relative to the control, whereas smoke ammonia from C4 was increased by 7.9 μg (33%), Table 6. The reasons are unclear.

The effects of the ingredients on smoke phenol yields were variable (Table 7). For many there was no significant effect relative to the control, some were decreased by up to 44%, especially with cigarettes C4 and C6, while a few were increased by up to 15%, especially with cigarette C9.

The effects of the ingredients on carbonyl compounds in smoke were also variable (Table 7). For several ingredient mixtures the levels were increased, formaldehyde in smoke from cigarette C3 was increased by 26 μg (73%) relative to the control and this increase remained significant when the long-term variability of the method is taken into account. This was the largest increase in any smoke analyte seen in Part I and Part II of this entire study. Smaller increases in smoke formaldehyde were also observed for cigarettes C5, C7, C8 and C9, and were also observed in cigarettes in Part I of the study (Baker et al., in press). One common ingredient in all these cigarettes, and in the relevant samples discussed in Part I, was sugar of one form or another (white sugar in C3, honey in C5, corn syrup in C7, sugar

Table 7
Experimental Cigarette Series C: phenolic and carbonyl compounds in smoke

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Phenols (μg/cig)									
Phenol	17.8	17.6	16.1[a]	10.0[a,b]	15.3[a]	12.9[a,b]	15.3[a]	15.5[a]	19.6[a]
Stand. Dev. ($n=5$)	0.278	1.32	0.616	0.627	1.41	0.337	0.821	1.06	0.879
m-+p-Cresol	12.1	12.4	12.2	8.48[a,b]	11.8	10.5[a,b]	11.7	11.8	13.7[a]
Stand. Dev. ($n=5$)	0.323	0.729	0.317	0.450	0.816	0.549	0.475	0.664	0.543
o-Cresol	4.14	4.14	4.20	2.81[a,b]	3.88	3.46[a,b]	3.99	3.98	4.75[a,b]
Stand. Dev. ($n=5$)	0.104	0.239	0.112	0.157	0.242	0.191	0.312	0.350	0.203
Catechol	50.5	53.0	52.0	44.2[a]	53.5[a]	49.4	52.7	52.5	54.5[a]
Stand. Dev. ($n=5$)	1.77	1.77	1.51	2.12	2.15	2.28	2.76	1.98	3.19
Hydroquinone	47.6	51.0	45.5	44.7	51.2[a]	46.8	47.7	47.5	50.2
Stand. Dev. ($n=5$)	2.27	1.19	1.11	1.66	1.95	2.78	2.58	2.29	1.78
Resorcinol	<1.1[c]	<1.1[c]	<1.1[c]	<1.1[c]	<1.1[c]	<1.1[c]	<1.1[c]	<1.1[c]	<1.1[c]
Carbonyls (μg/cig)									
Formaldehyde	35.6	36.3	61.6[a,b]	36.7	43.0[a,b]	30.2[a,b]	40.8[a]	45.0[a,b]	47.7[a,b]
Stand. Dev. ($n=5$)	2.81	0.890	5.20	1.77	1.25	1.51	2.21	1.32	1.86
Acetaldehyde	626	707[a]	649	695[a]	704[a]	659	708[a]	687[a]	663
Stand. Dev. ($n=5$)	21.8	38.2	36.5	24.8	39.8	30.2	27.0	22.8	37.6
Acetone	293	327[a]	325[a]	313[a]	335[a]	307	336[a]	329[a]	315
Stand. Dev. ($n=5$)	11.6	15.5	14.5	14.4	18.2	19.7	12.5	11.4	22.2
Acrolein	79.9	87.9[a]	88.1[a]	101[a,b]	95.9[a,b]	82.1	90.6[a]	85.0	83.4
Stand. Dev. ($n=5$)	4.50	4.95	5.34	3.52	5.41	3.84	4.89	3.54	6.27
Propionaldehyde	49.4	55.0[a]	51.5	55.0[a]	55.5[a]	55.5[a]	56.6[a]	53.6[a]	50.6
Stand. Dev. ($n=5$)	2.50	3.53	2.37	2.45	3.41	3.39	3.23	1.32	3.71
Crotonaldehyde	34.5	37.6[a]	37.8	34.7	37.1	34.1	38.3[a]	36.0	36.4
Stand. Dev. ($n=5$)	2.49	1.55	2.23	0.88	2.48	1.87	1.84	0.68	4.04
Methyl ethyl ketone	78.0	82.2	79.3	77.7	79.5	75.8	81.9	80.8	81.1
Stand. Dev. ($n=5$)	4.41	1.63	3.86	5.23	5.14	5.35	4.11	4.55	7.16
Butyraldehyde	36.8	40.6[a]	37.4	37.5	39.2	37.3	40.6[a]	39.4	37.2
Stand. Dev. ($n=5$)	2.39	1.32	2.33	1.83	2.22	2.85	2.33	1.49	3.88

[a] Significantly different to C1 (control) value at 95% confidence level—this data set.
[b] Also significantly different to C1 (control) value at 95% confidence level when long-term analytical method variability is included.
[c] Below detection limit of 1.1 μg/cig.

cane molasses in C8, invert sugar in C9, and brown sugar in B2 in Part I). Rustemeier et al. also observed increases in smoke formaldehyde yields of 60 and 65% relative to the control cigarette in two of their test cigarettes containing, inter alia, 4.17 and 6.25% corn syrup sugar respectively (Rustemeier et al., 2002; Carmines, 2002). They also observed a 16% increase in formaldehyde for a test cigarette containing invert sugar and sucrose amongst its ingredients. The likely formation of formaldehyde by the pyrolysis of sugars was discussed previously (Baker et al., in press). One exception to this generalisation is test cigarette C2, which contained high fructose corn syrup and yet had the same formaldehyde smoke yield as its control cigarette, C1.

The level of acetaldehyde was also elevated in test cigarettes C2, C4, C5, C7 and C8 by up to 78 μg (13%), Table 7, although these increases did not remain significant when the long-term variability of the analytical methodology was taken into account. Assuming these increases are due to the formation via pyrolysis of the ingredients, although some of these test cigarettes contain sugar ingredients (C2, C5, C7 and C8), sugar material per se is not the reason for increased smoke levels of acetaldehyde. Test cigarettes C3 and C9 contain up to 10.5% added sugar (Table 2) and produce smoke yields of acetaldehyde that are not statistically different to the control cigarette, C1 (Table 7). Furthermore, test cigarette B2 in Part I of the study (Baker et al., in press) had 6.2% of added brown sugar and the same smoke yield of acetaldehyde as its control cigartte. These findings are in agreement with recent reviews of the available data on acetaldehyde levels in smoke, which have concluded that sugars, including sucrose, D-fructose and D-glucose do not produce greater yields of acetaldehyde in smoke than are produced from tobacco itself on a weight-for-weight basis (Seeman et al., 2002, Paschke et al., 2002, Sanders et al., 2003).

For the ingredient malic acid, used in cigarette C4, detailed pyrolysis data have recently been obtained when the substance was pyrolysed under heating conditions that simulate those in a burning cigarette (Baker and Bishop, in press). The pyrolysis results indicated that the malic acid completely decomposed and, at its maximum application level in a cigarette (6500 ppm), it was calculated that it could yield a maximum of 330 μg of acetaldehyde in the cigarette smoke. The calculation

Table 8
Experimental Cigarette Series C: miscellaneous organic compounds, benzo[*a*]pyrene, semi-volatile bases and aromatic amines in smoke

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Compound (μg/cig)									
1,3-Butadiene	30.0	31.5	28.7	29.5	31.2	30.9	30.8	29.0	29.1
Stand. Dev. ($n=5$)	2.07	2.97	1.56	2.14	3.46	1.78	1.97	0.77	1.22
Isoprene	410	405	392[a]	432[a]	428	426	406	401	398
Stand. Dev. ($n=5$)	11.2	17.4	12.1	2.51	29.9	14.5	21.4	10.5	11.8
Acrylonitrile	17.5	17.7	16.2[a]	17.2	17.4	17.0	16.4[a]	16.5[a]	16.9
Stand. Dev. ($n=5$)	0.625	0.501	0.497	0.524	0.538	0.811	0.514	0.378	0.929
Benzene	56.0	55.2	55.0	57.4	57.5	54.9	54.2	55.7	56.7
Stand. Dev. ($n=5$)	0.99	1.49	0.85	1.41	1.75	1.91	2.11	1.76	1.44
Toluene	107	110	103[a]	108	110	105	105	108	110
Stand. Dev. ($n=5$)	2.10	2.44	0.58	3.40	3.20	2.79	2.91	2.23	2.15
Styrene	13.5	13.9	12.1	13.7	13.7	13.3	13.2	13.4	13.8
Stand. Dev. ($n=5$)	1.11	0.667	0.915	0.791	0.749	0.823	0.739	0.484	0.423
Benzo[a]pyrene (ng/cig)	13.6	13.5	14.4	13.8	14.5	12.7	13.3	13.2	13.3
Stand. Dev. ($n=5$)	0.727	1.18	1.06	0.969	0.843	1.05	1.18	1.12	1.26
Bases (μg/cig)									
Pyridine	15.2	15.5	13.5[a]	14.0[a]	14.4[a]	14.1[a]	14.8	14.7	15.2
Stand. Dev. ($n=5$)	0.41	1.00	0.54	0.94	0.61	0.91	0.94	0.41	0.63
Quinoline	0.63	0.60	0.63	0.53[a]	0.64	0.62	0.55[a]	0.54[a]	0.60
Stand. Dev. ($n=5$)	0.061	0.082	0.080	0.028	0.069	0.068	0.047	0.023	0.061
Amines (ng/cig)									
1-Aminonaphthalene	14.6	16.0[a]	11.7[a]	11.6[a]	12.4[a]	12.2[a]	13.2[a]	12.4[a]	13.9
Stand. Dev. ($n=5$)	0.595	0.846	0.435	0.614	0.671	0.801	0.504	0.590	0.738
2-Aminonaphthalene	9.94	11.5[a]	7.67[a,b]	8.07[a,b]	8.58[a]	8.30[a]	9.11[a]	8.53[a]	9.68
Stand. Dev. ($n=5$)	0.326	0.567	0.437	0.306	0.661	0.429	0.219	0.208	0.402
3-Aminobiphenyl	3.94	4.36[a]	3.05[a,b]	3.40[a]	3.41[a]	3.41[a]	3.58[a]	3.40[a]	3.70
Stand. Dev. ($n=5$)	0.177	0.266	0.199	0.111	0.217	0.137	0.154	0.144	0.152
4-Aminobiphenyl	2.25	2.48[a]	1.67[a,b]	1.96[a]	1.98[a]	2.02[a]	2.06[a]	1.94[a]	2.14
Stand. Dev. ($n=5$)	0.104	0.097	0.142	0.086	0.153	0.117	0.114	0.068	0.081

[a] Significantly different to C1 (control) value at 95% confidence level—this data set.
[b] Also significantly different to C1 (control) value at 95% confidence level when long-term analytical method variability is included.

was for an unfiltered (plain) cigarette with the maximum ingredient application level, maximum tobacco weight in the cigarette, maximum proportion of tobacco burnt in puffing and maximum transfer to mainstream smoke. This maximum level of malic acid was added to the test cigarette C4 in the present study (Table 2) and an increased level of only 69 μg of acetaldehyde was found in the smoke relative to the control (Table 7), and this increase was not significant when the long-term variability of the analytical methodology was taken into account. This indicates either that the maximum calculated pyrolysis yields in smoke (Baker and Bishop, in press) were greatly over-estimated, or that complete pyrolysis of the malic acid does not occur in the burning cigarette, or that the generation of acetaldehyde from malic acid is not too much higher than generation from the tobacco that it replaces in the cigarette, on a *per* weight basis.

The level of acrolein in smoke was also elevated relative to the control cigarette for five of the test cigarettes, by 21.1 μg (26%) for test cigarette C4 (Table 7). This cigarette has, inter alia, 7.0% added glycerol (Table 2). Based on a published pyrolysis study (Stein and Antal, 1983) it could be tempting to speculate that the increased acrolein in smoke is formed from the glycerol. Stein and Antol showed that acrolein and acetaldehyde were the principal products from the isothermal pyrolysis of glycerol in steam at temperatures above 650 °C, and at lower temperatures in the presence of a metal catalyst. However, the pyrolysis conditions used by Stein and Antol were not the same as those that occur inside the burning zone of a cigarette during smoking. Many studies have shown that if the pyrolysis conditions do not simulate those that occur during tobacco combustion, false predictions can be made if they are used to calculate the behaviour of the substance in a burning cigarette, as reviewed by Baker and Bishop (in press). In fact, when glycerol was pyrolysed under conditions that were designed to simulate burning cigarette conditions during smoking, Baker and Bishop demonstrated that 99.8% of the glycerol (boiling point 290 °C) was found intact in the pyrolysate, and neither acrolein

Table 9
Experimental Cigarette Series C: metals in tobacco blend and smoke yields

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Blend (ng/g)									
Pb	811	773	848	827	945[a]	934[a]	820	975[a]	812
Stand. Dev. ($n=5$)	59.7	36.5	38.8	86.7	56.0	69.5	43.6	108	30.6
Cr	1070	1510[a,b]	1500[a,b]	1360[a]	1760[a,b]	1610[a,b]	1180	2130[a,b]	1530[a,b]
Stand. Dev. ($n=5$)	94.7	86.7	109	117	125	154	122	178	165
Ni	1960	3040[a,b]	1960	3370[a,b]	3400[a,b]	3410[a,b]	3100[a,b]	2870[a,b]	2140
Stand. Dev. ($n=5$)	204	250	189	201	301	264	281	188	181
Cd	1086	1050	940[a]	1040	1010	992[a]	1040	1000	1025
Stand. Dev. ($n=5$)	58.4	63.8	90.1	64.9	116	28.6	92.8	77.9	50.2
Hg	24	24	22	23	23	24	23	23	23
Stand. Dev. ($n=5$)	1.6	2.3	2.2	3.0	2.2	2.4	3.6	2.9	2.2
As	304	332	271	264	282	287	338	271	259[a]
Stand. Dev. ($n=5$)	25.0	32.0	37.5	36.3	37.5	32.4	56.7	41.9	33.4
Se	<105	<105	<105	<105	<105	<105	<105	<105	<105
Smoke (ng/cig)									
Pb	35	32	40	42[a]	38	37	39	39	41
Stand. Dev. ($n=5$)	4.1	1.5	4.8	4.3	1.9	2.2	8.3	3.9	4.2
Cr	<3	<3	<3	<3	<3	<3	<3	<3	<3
Ni	<12	<12	<12	<12	<12	<12	<12	<12	<12
Cd	58	51	53	58	56	52	53	60	63
Stand. Dev. ($n=5$)	6.7	3.9	5.2	2.6	5.5	2.7	6.6	3.3	4.2
Hg	4.0	4.2	4.6	3.9	4.8	3.7	3.8	3.9	3.9
Stand. Dev. ($n=5$)	0.30	0.34	0.94	0.29	0.91	0.32	0.28	0.16	0.20
As	<15	<15	<15	<15	<15	<15	<15	<15	<15
Se	<20	<20	<20	<20	<20	<20	<20	<20	<20

< Indicates that the level is below detection or quantitation limit.
[a] Significantly different to C1 (control) value at 95% confidence level—this data set.
[b] Also significantly different to C1 (control) value at 95% confidence level when long-term analytical method variability is included.

nor acetaldehyde were detected. These pyrolysis results were in agreement with unpublished results quoted by the Philip Morris tobacco company [Philip Morris USA, 2001 (unpublished report—can be obtained from the corresponding author upon request). These unpublished results were obtained by heating glycerol in air up to 900 °C in 100 s, and two studies on smoking cigarettes in which radiolabelled glycerol had been added. In all cases the glycerol distilled intact and the presence of acrolein and acetaldehyde from the glycerol was very small, e.g. less than 0.1% acrolein in the radiolabelled mainstream smoke products. Consequently, published studies using pyrolysis conditions different to those occurring inside a burning cigarette, such as those of Stein and Antol (1983), are not relevant to a burning cigarette and are not considered further in the present paper. The implications of the pyrolysis studies relevant to burning cigarette conditions (Baker and Bishop, in press), that acrolein is not formed from glycerol, are partly supported by other studies in which glycerol was added to a cigarette and the effect on smoke chemistry determined. Thus in their literature review, Paschke et al. (2002) quote a 1977 US National Cancer Institute report that indicated a 7.9%, probably not statistically significant, increase in smoke acrolein when 2.95% glycerol was added to tobacco. Rustemeier et al. reported 13% increases in smoke acrolein in smoke from cigarettes containing glycerol and other ingredients, for test cigarettes containing both 2.8 and 4.2% glycerol, i.e. no dose response was observed, implying glycerol was not the precursor of acrolein (Rustemeier et al., 2002; Carmines, 2002). However, the acrolein levels in test cigarette C4 are elevated in the present study and further studies would be needed to investigate the specific mechanistic reasons.

Of course, all this begs the question as to what ingredients were responsible for the increased acrolein in smoke. Test cigarettes C8, C9 (Table 7) and B2 (Baker et al., in press) each contained up to 7.0% sugar and yet had no elevated smoke acrolein levels relative to their controls (Table 7). This suggests that the increased acrolein was not formed from sugar substances, and studies reviewed by Paschke et al. (2002) support this conclusion. The responsible ingredient(s) from the remaining list of ingredients in Table 2 for cigarettes C2, C3, C4, C5 and C7 (that all led to higher smoke acrolein levels) cannot be deduced further with the present data.

A few of the miscellaneous "Hoffmann organic analytes" in smoke were decreased by up to 7% in smoke relative to the control, isoprene for cigarette C4 was increased by 5%, while most were not significantly different to the control (Table 8). None of these differences

Table 10
Significant smoke "Hoffmann analyte" increases and long-term analyte variability

Cig. code	Analyte	Unit	Levels in smoke				Variability (±2 s.d.[a])	Is diff. > variability?
			Control	Test	Diff.	% Diff.		
A7	TPM	mg	17.3	18.6	1.3	7.5	1.97	No
	NFDPM	mg	12.8	13.5	0.7	5.5	1.26	No
A8	TPM	mg	17.3	18.9	1.6	9.2	1.97	No
	NFDPM	mg	12.8	13.9	1.1	8.6	1.26	No
B2	HCN	μg	209	260	51	24	38.0	Yes
	Formaldehyde	μg	23.8	29.5	5.7	24	5.32	Yes
B3	TPM	mg	18.6	20.4	1.8	9.7	2.12	No
	NFDPM	mg	14.2	15.5	1.3	9.2	1.40	No
	CO	mg	14.2	15.2	1.0	7.0	1.75	No
B5	Formaldehyde	μg	23.8	39.9	16.1	68	5.32	Yes
	Acrolein	μg	72.2	93.3	21.1	29	15.8	Yes
	Propionaldehyde	μg	54.1	66.2	12.1	22	10.2	Yes
C3	Formaldehyde	μg	35.6	61.6	26	73	7.96	Yes
C4	TPM	mg	18.6	20.5	1.9	10	2.12	No
	NFDPM	mg	13.3	14.3	1.0	7.5	1.31	No
	NH_3	μg	24.0	31.9	7.9	33	6.25	Yes
	Acrolein	μg	79.9	101	21.1	26	17.4	Yes
C5	Formaldehyde	μg	35.6	43.0	7.4	21	7.96	No
	Acrolein	μg	79.9	95.9	16	20	17.4	No
C8	Formaldehyde	μg	35.6	45.0	9.4	26	7.96	Yes
C9	HCN	μg	238	274	36	15	43.3	No
	o-Cresol	μg	4.14	4.75	0.61	15	0.768	No
	Formaldehyde	μg	35.6	47.7	12.1	34	7.96	Yes

NFDPM is nicotine-free dry particulate matter, which is commonly known as "tar".

[a] Obtained from standard deviation of historical data on the 1R4F reference cigarette for each smoke analyte, determined in the analytical laboratory of the present study, and assuming a constant coefficient of variation between the 1R4F and control cigarettes.

remained significant when the long-term variability of the method was taken into account.

The yields of benzo[*a*]pyrene in smoke from cigarettes C2–C9 were not significantly different to the yield in the control, C1 (Table 8). The yield of the "Hoffmann semi-volatile bases", pyridine and quinoline, were either not significantly different to the control, or were reduced by up to 16%.

The effects of the ingredients on the "Hoffmann aromatic amines" were variable (Table 8). The ingredient mixture in cigarette C2 produced increases of up to 16% relative to the control in all four amines, although none of these increases remained significant when the long-term variability of the method was taken into account. The ingredient mixtures in cigarettes C3–C8 produced decreases in the aromatic amines in smoke of up to 26% and some of these differences remained significant even when the long-term variability of the method was taken into account. The ingredient mixtures used in cigarette C9 had no significant effect on the level of any of the aromatic amines in smoke relative to the control cigarette, C1.

The presence of all of the ingredient mixtures on almost all of the test cigarettes increased the level of chromium in the tobacco, almost doubling it for cigarette C8 (Table 9). Increases up to 74% were also found for nickel in the tobacco for most of the test cigarettes. Presumably these metals were present as contaminates in the ingredient mixtures. However, these increased levels were not detected in the smoke yields, although the chromium and nickel yields in smoke were below their levels of detection or quantitation for all the cigarettes. Only the level of lead in the smoke from cigarette C4 was elevated relative to the control, and that increase was not statistically significant when the long-term variability of the analytical methodology was taken into account. All other metal levels were not significantly different to the control.

Table 11
Carbonyl re-analyses of results where test/control differences > long-term variability

Cig. code	Analyte	Unit	Analysis place/No.[a]	Levels in smoke			
				Control	Test	Diff.	% Diff.
B5	Formaldehyde	μg	Brazil 1	23.8	39.9[b,c]	16.1	68
			UK 1	29.7	50.7[b,c]	21.0	71
	Acrolein	μg	Brazil 1	72.2	93.3[b,c]	21.1	29
			UK 1	78.0	91.6[b]	13.6	17
	Propionaldehyde	μg	Brazil 1	54.1	66.2[b,c]	12.1	22
			UK 1	55.0	62.4[b]	7.4	13
C3	Formaldehyde	μg	Brazil 1	35.6	61.6[b,c]	26.0	73
			Brazil 2	42.3	68.4[b,c]	26.1	62
			UK 1	42.5	76.1[b,c]	33.6	79
C4	Acrolein	μg	Brazil 1	79.9	101[b,c]	21.1	26
			Brazil 2	85.5	94.4[b]	8.9	10
			UK 1	77.9	89.4[b]	11.5	15
C8	Formaldehyde	μg	Brazil 1	35.6	45.0[b,c]	9.4	26
			Brazil 2	42.3	53.7[b,c]	11.4	27
			UK1	42.5	57.1[b,c]	14.6	34
C9	Formaldehyde	μg	Brazil 1	35.6	47.7[b,c]	12.1	34
			UK 1	42.5	55.2[b,c]	12.7	30

[a] Brazil 1—Analysed in BAT (Souza Cruz), Brazil, February 2002; Brazil 2—Analysed in BAT (Souza Cruz), Brazil, March 2003; UK 1: Analysed in BAT, UK, April 2003.
[b] Significantly different to C1 (control) value at 95% confidence level—this data set.
[c] Also significantly different to C1 (control) value at 95% confidence level when long-term analytical method variability is included.

4. Significance of smoke chemistry increases

A key requirement of the present study was to determine which of the ''Hoffmann analytes'' might be increased in smoke by the use of tobacco ingredients. In Section 3 above we have highlighted those analytes that were increased and suggested possible chemical reasons for their increase. From all of the results in both Parts I and II of this study, those ''Hoffmann analyte'' smoke yields that are, statistically, significantly higher than those from the relevant control cigarette are listed in Table 10, calculated using the pro rata long-term standard deviations of the 1R4F reference cigarette, as described in Section 3 above. The percentage differences between test and control cigarettes range from 5.5 to 73%, although in some cases the absolute differences are small. Given the high overall long-term variabilities in the results, which of the differences between the control and test cigarettes are 'real', i.e. which would still be present if the analyses were repeated? Over a large sample set, ± 1.96 standard deviations would give a 95% confidence limit of the total variability in a given smoke analyte level (White et al., 1979). If the difference between control and test levels is greater than this total variability then the difference was considered to be 'real'. The increases concluded to be 'real' are indicated in Table 10. Thus the formaldehyde increases for test cigarettes B2, B5, C3, C8 and C9 are 'real', as are the acrolein increases for test cigarettes B5 and C4, the propionaldehyde increase for B5, the HCN increase for B2, and the NH_3 increase for C4. For cigarette series A from Part I of this study (Baker et al., in press), where flavouring ingredients were added to a typical US blended tobacco, it is seen that none of the increases of smoke analytes in the test cigarettes relative to their control were 'real'.

In order to confirm that the differences indicated in Table 10 are actually 'real', the test cigarettes that indicate 'real' increases in carbonyl compounds relative to their control cigarette have been re-analysed. The re-analyses were done more than a year after the original analyses, in some cases at both the original British American Tobacco analytical laboratory in Brazil and also at the British American Tobacco laboratory in the UK. The results, shown in Table 11, generally confirm that the repeat analyses produced similar relative increases in the level in the smoke from the test cigarette relative to the control cigarette. The absolute levels in smoke were, however, often different, as would be expected from the general long-term variability of the analytical methodology. For completeness, the re-analyses of some of the smoke carbonyl compounds for test cigarette C5, which did not indicate 'real' differences relative to its control cigarette (C1) in Table 10, are given in Table 12. Some of the repeat analyses did indicate significant differences to the control cigarette on the

Table 12
Re-analyses of carbonyls where test/control differences < long-term variability

Cig. code	Analyte	Unit	Analysis place/No.[a]	Levels in smoke			
				Control	Test	Diff.	% Diff.
C5	Formaldehyde	μg	Brazil 1	35.6	43.0[b,c]	7.4	21
			Brazil 2	42.3	48.4[b]	6.1	14
			UK 1	42.5	50.7[b]	8.2	19
	Acrolein	μg	Brazil 1	79.9	95.9[b,c]	16	20
			Brazil 2	85.5	81.4	−4.1	−4.8
			UK 1	77.9	87.2[b]	9.3	12

[a] Brazil 1, analysed in BAT (Souza Cruz), Brazil, February 2002; Brazil 2, analysed in BAT (Souza Cruz), Brazil, March 2003; UK 1, analysed in BAT, UK, April 2003.
[b] Significantly different to C1 (control) value at 95% confidence level—this data set.
[c] Also significantly different to C1 (control) value at 95% confidence level when long-term analytical method variability is included.

Table 13
Comparison of formaldehyde yields in smoke from test, control and flue-cured reference cigarette

Cigarette	Formaldehyde (μg/cig)
B1 (control)	23.8
B2	29.5
B5	39.9
Ref2[a]	38.6
C1 (control)	35.6
C3	61.6
C8	45.0
C9	47.7
Ref2[a]	42.5

[a] BAT internal reference cigarette. 100% flue-cured tobacco, no added tobacco ingredients.

occasion of the repeat measurement, but not when the pro rata standard deviation from the long-term variability was included. Furthermore, on the occasion of the repeat analysis for acrolein in the Brazilian laboratory, there was no statistically significant difference to the control. Thus, the use of this somewhat pragmatic approach for determining whether or not differences in smoke yields are 'real' does produce repeat results that are in broad agreement.

In the present smoke analysis study, the smoke yields of the 1R4F reference cigarette and two internal British American Tobacco reference cigarettes were also determined. One of these reference cigarettes, designated Ref2, contained 100% flue-cured tobacco and no added tobacco ingredients (Baker et al., in press). The formaldehyde smoke levels from this reference cigarette, determined at the same time as the test cigarettes B2 and B5, and C3, C8 and C9, are compared in Table 13. It is seen that the smoke formaldehyde level of the Ref2 and B5 test cigarettes are very similar. Thus the addition of the cellulose and other polysaccharide ingredients to the all-tobacco US tobacco blend in B1 produced a cigarette whose smoke had formaldehyde levels similar to a flue-cured cigarette with no added ingredients. Flue-cured tobacco contains relatively high levels of sugars naturally present in the tobacco, produced from tobacco starch during the flue-curing process (Leffingwell, 1999). US blended tobacco (as used in cigarettes B1 and B5) contains relatively low levels of naturally-present sugars. Thus the sugars naturally present in a flue-cured tobacco cigarette (Ref2) produce the same levels of formaldehyde in smoke as the cellulosic and polysaccharide ingredients added to the tobacco-only US blended cigarette (B5). Control cigarette C1 gave a higher yield of smoke formaldehyde than control cigarette B1, because the tobacco in C1 contained some cellulose in the reconstituted sheet included in the US tobacco blend (Table 1). The addition of sugar in the casing (test cigarette C3, Table 2) gave smoke with a substantially higher formaldehyde yield than that from the flue-cured reference cigarette Ref2 (Table 13).

We have dealt in some detail with the significance of these smoke chemistry results because there are a small number of statistically significant increases in the presence of some ingredients, in particular formaldehyde levels from sugars and cellulose. These increases are similar to those reported in the Philip Morris study by Rustemeier et al. (2002) and some of the studies included in the review of Paschke et al. (2002). We repeat the statement of Rustemeier et al. (2002) that further research is warranted in order to investigate these differences further. However, and again similar to the overall findings in the Philip Morris study (Carmines, 2002), these chemical differences have not resulted in any differences in the various bioassays undertaken, as summarised in an overview paper of these results (Baker et al., submitted for publication).

5. Overall summary and conclusions

1. In this part of the study the effects of 29 casing flavour ingredients and three humectants on the yields of the 44 "Hoffmann analytes" in cigarette smoke have been assessed. The ingredients were added in various mixtures to eight test cigarettes at their maximum use level, up to a maximum of 68 mg, and the yield of the analytes in smoke compared to a control cigarette. This brings the total number of ingredients assessed in Parts I and II of the study to 482, comprising 462 flavours, one flavour/solvent, one solvent, seven preservatives, five binders, three humectants, one filler and two process aids (one of which is water).
2. The presence of many of the ingredient mixtures either had no significant effect on the levels of the "Hoffmann analytes" in smoke, or they produced decreases relative to a control cigarette, up to 44% in some cases. The important consideration in this study was whether or not tobacco ingredients increase, in a 'real' sense, the levels of "Hoffmann analytes" in smoke, i.e. whether any increases would be seen on repeat analyses. A pragmatic consideration of the long-term variability of the smoke chemistry analyses suggests that only a small number of analytes fall into this category. These are detailed below.
3. Propylene glycol (a humectant) and water (a process aid) were common to all the ingredient mixtures. One test cigarette (C6) contained only these two ingredients. With this cigarette almost all of the "Hoffmann analytes" in smoke were either not statistically different to the control, or had their levels reduced by up to 28%. Only the level of propionaldehyde was increased in smoke, by 6.1 μg (12%), and this increase was not significant when the long-term variability of the analytical methodology was taken into account.
4. The amount of TPM in the smoke from the test cigarette (C4) with glycerol in the ingredient mixture was increased by 1.9 mg (10%) relative to the control. This was most probably caused by the direct intact transfer of glycerol to the smoke particulate phase.
5. The smoke level of NH_3 in cigarette C4 was increased by 7.9 μg (33%).
6. The level of formaldehyde was increased in virtually all the cigarettes that contained sugars in the casing mixture, in one case (cigarette C3) by 26 μg (73%). This was the largest increase in any smoke analyte seen in the entire study. Other studies have shown that formaldehyde is formed during the pyrolysis of sugars.
7. The levels of acrolein were elevated in smoke from some of the test cigarettes, by 21 μg (26%) relative to the control cigarette for one test cigarette (C4) with added glycerol. Consideration of the present results, together with information from the pyrolysis of glycerol under conditions relevant to those in a burning cigarette, suggest that the increased acrolein was not generated from reaction of glycerol or sugars.

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