The Characterization of Cigarette Smoke from Cytrel® Smoking Products* and its Comparison to Smoke from Flue-Cured Tobacco

III. Particulate Phase Analysis*

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INTRODUCTION

This paper describes the analysis of the particulate phase of smoke from cigarettes containing Cytrel, flue-cured tobacco, and Cytrel-tobacco blends. The particulate phase is defined as the relatively non-volatile material from smoke. It is generally obtained by drawing cigarette smoke through Cambridge filter pads, impaction traps, or gas traps cooled to cryogenic temperatures. The composition of approximately 70 % of the particulate phase from mainstream Cytrel smoke has been determined. A considerably smaller portion of the particulate phase from mainstream tobacco smoke has been similarly defined due to its greater complexity.

EXPERIMENTAL

Cigarette Manufacture and Smoking Procedure

The physical characteristics of the cigarettes and the smoking procedures used in this study were described in the first paper in this series (1). For this work three types of smoking machines were used. Below is a list of the analyses done with each type of smoking machine.

Piston-action smoking machine	Keith-Newsome smoking machine	Mason 20-port smoking machine
Phenolic compounds	Cambridge particulate matter	Polycyclic aromatic hydrocarbons ^a
Benzo(a)- pyrene	Water	Elemental analysis ^b
Nitrosamines	Nicotine	
	Glycerol	
	pH	

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* Cytrel® is a registered trademark of Celanese Corporation.

Analytical Procedures

Cambridge Particulate Matter, Water, and Nicotine: Cambridge particulate matter (CPM), water, and nicotine deliveries were determined according to a protocol established in a study by Ogg and Shultz (2). CPM was measured gravimetrically. Water and nicotine were measured by first extracting CPM with a known volume of isopropanol containing ethanol as an internal standard. The water concentration of the extract was determined by gas chromatography. Nicotine was determined spectrophotometrically after it was steam-distilled from an aliquot of the isopropanol extract (3). Particulate matter (water and nicotine free), PM(WNF), was calculated by subtracting the water and nicotine deliveries from CPM.

Glycerol: Glycerol was determined in the isopropanol extract of CPM by gas chromatography using a 4 ft. × 1/8 in. stainless steel column filled with 60/80 mesh Tenax-GC and maintained at 205 °C.

pH: The pH of smoke was determined according to the method of Artho and Grob (4).

Phenolic Compounds: Phenolic compounds were extracted from smoke condensate and concentrated according to the method of Hoffmann and Wynder (5). Mainstream smoke was collected in a trap filled with glass wool and cooled to -78 °C. The tar was washed from the trap with 5 % NaOH and o-chlorophenol was then added as an internal standard. After basic contaminants were extracted from the caustic solution using ether, the phenolic compounds were regenerated from the salt form by acidification and extracted with ether. The ether fraction containing the phenolic compounds was concentrated to approximately 3 ml and dried over anhydrous Na₂SO₄. The fraction was then analyzed

a: Condensate prepared by Inveresk Research International, Musselburgh, Scotland, and Battelle Genève, Geneva, Switzerland.

b: Condensate prepared by B. Frost of Carreras Rothmans Limited, London, England.

Figure 1. Typical chromatograms from the determination of phenolic compounds.

by gas chromatography at 235 °C using a 13 ft. $\times$ 1/8 in. stainless steel column filled with 80/100 mesh Chromosorb 101. Typical chromatograms of this analysis for Cytrel and flue-cured tobacco are shown in Figure 1.

Polycyclic Aromatic Hydrocarbons:

a) Single component analysis — benzo(a)pyrene: Benzo(a)pyrene (BaP) was determined according to the procedure described by Davis, Lee, and Davidson (6) with the addition of a thin layer chromatographic step using ChromAR sheet prior to the paper chromatography.

b) Multicomponent analysis: This survey analysis was used for the determination of several polycyclic aromatic hydrocarbons (PAH) in smoke condensate. The procedure is a combination of techniques which have been described in the literature (6–12) and is most closely modelled after that of Zane (7). An outline of the procedure appears below.

1. Dissolve condensate in 6:1 methanol/water.
2. Extract with hexane; discard methanol/water.
3. Extract with acetonitrile; discard hexane.
4. Gel chromatography on Sephadex LH-20 with isopropanol as the mobile phase.

5. Thin layer chromatography on ChromAR sheet with 9:1 hexane : benzene as the mobile phase.

6. Gas chromatography on 5% Dexsil 300/Chromosorb W (20 ft. $\times$ 1/8 in. stainless steel column held at 165 °C for 8 minutes, programmed from 165° to 295 °C at 1°/min, and held at 295 °C for 60 min).

Individual PAH were identified by GC retention times of PAH standards and by mass spectrometry. Quantitation was accomplished with computerized integration or peak height measurements. Although 16 PAH were qualitatively detected, quantitative results were reported only for those compounds whose GC peaks were shown by mass spectrometry to be of the order of 99 % pure.

The PAH results have been corrected for losses which occurred during analysis. The degree of recovery of each PAH was determined by the concurrent analysis of an unadulterated sample of condensate and a sample to which known amounts of PAH had been added. Since no sample bias was observed, the average degree of recovery for each PAH from 25 determinations (including some samples not reported here) was calculated. The average recoveries ranged from 64 to 87 %.

A chromatogram of an artificial mixture of PAH standards is shown in Figure 2, and typical chromatograms of a 100 % Cytrel smoke condensate and a 100 % tobacco smoke condensate are shown in Figure 3.

Steam-Volatile Nitrosamines: Dimethylnitrosamine (DMN) and N-nitrosopyrrolidine (NNP) are the two steam-volatile nitrosamines which occur in the greatest concentrations in cigarette smoke (13). These nitrosamines have been determined by a procedure which is based on a combination of analytical techniques previously reported (13–18). The procedure is closely modelled after that described by Baylis and Underwood (13) except that a mass spectrometer of lower resolution has been substituted. The potential loss of specificity resulting from the lower resolution has, however, been obviated by improved chromatographic resolution prior to mass spectrometric detection. An outline of the procedure is given below.

1. Collect mainstream smoke in a trap cooled with dry ice.
2. Steam-distil directly from the trap.
3. Extract distillate with methylene chloride and concentrate by solvent evaporation.
4. Thin layer chromatography on silica gel using 5:7:10 hexane : diethyl ether : methylene chloride as the mobile phase.
5. Recover DMN and NNP from the TLC plate using methylene chloride containing dipropylnitrosamine (DPN) as an internal standard.
6. Gas chromatography with a 150 ft. $\times$ 0.01 in. open tubular stainless steel column coated with Emulphor ON and programmed from 75 to 195 °C at 2°/min.

Figure 2. Chromatographic separation of a mixture of polycyclic aromatic hydrocarbon standards.

Figure 3. Typical chromatograms from the determination of polycyclic aromatic hydrocarbons.

Figure 4. Typical chromatograms from nitrosamine determination (flame ionization detector).

The effluent from the GC column was split and routed to three separate detectors — a flame ionization detector, a nitrogen-selective detector, and a differentially pumped mass spectrometer. The first two detectors were used for survey purposes while the third was used for quantitation. The mass spectrometer was tuned to m/e 74 for DMN, m/e 130 for DPN, and m/e 100 for NNP. The particular m/e values were chosen to obtain maximum specificity and sensitivity. Typical chromatograms for Cytrel and tobacco using the three detectors are shown in Figures 4–6.

Quantitation was accomplished by peak height mea-

Figure 5. Typical chromatograms from nitrosamine determination (nitrogen-selective detector).

surement with reference to calibration factors determined on known nitrosamine concentrations. The results reported in Table 1 have been corrected for losses which occurred during analysis. These losses were estimated by analyzing each sample unadulterated and spiked with DMN and NNP standards equivalent to 20 ng/cigarette. Recoveries were generally near 20 % but varied considerably between determinations. Since no sample bias was evident and recoveries appeared to vary randomly, average recoveries based on 46 determinations (including analyses on samples not reported here) were calculated. The average recovery for DMN was 19.4 % ($\sigma = 10.4$ %) and for NNP was 13.7 % ($\sigma = 8.3$ %).

A further complication to this analysis is the possibility of producing nitrosamine artefacts in the cold traps as

Figure 6. Typical chromatograms from nitrosamine determination (mass spectrometer "detector").

condensate is collected (19, 20). However, since the nitrosamine deliveries are low and quite variable for all of the samples examined, the possibility of the artefact formation of DMN and NNP does not alter the conclusions drawn from a comparison of deliveries from Cytrel and tobacco blend cigarettes.

Elemental Analyses: Accu-Labs Research, Inc., Wheat Ridge, Colorado, performed the survey elemental analysis on smoke condensate by spark source mass spectrography and the determination of mercury by a combustion-amalgamation technique followed by atomic fluorescence spectroscopy. Special precautions were taken in the generation of the condensate samples to insure that no ash was transferred to the tar.

RESULTS AND DISCUSSION

The results of the particulate phase analyses of smoke from cigarettes made from Cytrel, flue-cured tobacco, and blends of the two are shown in Tables 1 and 2. Each result is the average of at least two determinations.

Comparison of 100 % Cytrel and 100 % Tobacco Cigarettes

Considering only the data in Table 1 for 100 % Cytrel and 100 % tobacco cigarettes, it can be seen that the deliveries of particulate matter, nicotine, phenols, polycyclic aromatic hydrocarbons, and nitrosamines were all lower from Cytrel than from tobacco cigarettes. Only one compound, glycerol, was delivered in greater

Table 1. Particulate phase analyses of smoke from cigarettes containing blends of tobacco with Cytrel®.

Test	Sample				
	100 % tobacco	10 % Cytrel	20 % Cytrel	50 % Cytrel	100 % Cytrel
1. Cambridge particulate matter: (mg/cigarette)	20.6	18.1	17.7	13.0	5.2
a. Water (mg/cigarette)	3.7	2.9	4.3	3.2	1.6
b. Nicotine (mg/cigarette)	1.1	0.9	0.9	0.4	Nil
c. PM (WNF)* (mg/cigarette)	15.8	14.3	12.5	9.4	3.6
2. Glycerol (mg/cigarette)	<0.1	0.2	0.4	1.3	2.1
3. pH	5.7	5.8	5.9	6.4	8.1
4. Phenolic compounds (µg/cigarette):					
Phenol	52	44	36	16	<0.5
o-Cresol	14	16	14	8	<0.5
m- and p-Cresol	27	22	20	10	<0.5
o-Methoxyphenol	8	7	6	3	<0.5
2,3-Dimethylphenol	2	2	1	<0.5	<0.5
2,4- and 2,5-Dimethylphenol	9	9	6	5	<0.5
2,6-Dimethylphenol	5	7	5	2	<0.5
3,4-Dimethylphenol	2	2	2	1	<0.5
p-Ethyl- & 3,5-dimethylphenol	11	10	7	5	<0.5
o-Ethylphenol	4	3	3	2	<0.5
2,3,5-Trimethylphenol	<0.5	<0.5	<0.5	<0.5	<0.5
2,4,6-Trimethylphenol	<0.5	<0.5	<0.5	<0.5	<0.5
5. Polycyclic aromatic hydrocarbons (ng/cigarette):					
<i>Specific analysis:</i>					
Benzo(a)pyrene	20	20	13	10	4
<i>Survey analysis:</i>					
Phenanthrene & anthracene	370	300	310	220	22
3-Methylphenanthrene	200	160	120	100	6
Fluoranthene	130	110	92	76	19
Pyrene	91	70	56	47	9
Benzfluoranthene	21	20	14	14	4
Benzo(a)pyrene & benzo(e)pyrene	20	17	13	10	5
6. Steam-volatile nitrosamines (ng/cigarette):					
Dimethylnitrosamine	21	34	18	21	ND**
N-Nitrosopyrrolidine	29	15	11	ND	ND

* PM (WNF) = Particulate matter (water and nicotine free).

** ND = Not detected.

Table 2. Spark source mass spectrographic* analysis of particulate matter from cigarettes containing blends of tobacco with Cytrel®.

Element	100 % tobacco	50 % Cytrel	100 % Cytrel
Aluminum	52	12	1.7
Antimony	<0.9	<0.5	<0.1
Arsenic	21	4.6	0.07
Barium	0.5	0.7	0.2
Beryllium	<0.05	<0.03	<0.007
Bismuth	<0.4	1.7	0.2
Boron	NR**	NR	NR
Bromine	150	19	0.23
Cadmium	250	14	0.53
Calcium	150	45	11
Carbon	NR	NR	NR
Cerium	0.5	0.4	0.07
Caesium	—	—	—
Chlorine	5900	80	11
Chromium	1.1	0.2	0.2
Cobalt	0.1	0.07	<0.004
Copper	27	7.7	0.2
Dysprosium	—	—	—
Erbium	—	—	—
Europium	—	—	—
Fluorine	45	11	20
Gadolinium	—	—	—
Gallium	0.2	0.1	<0.02
Germanium	1.1	0.06	0.01
Gold	—	—	—
Hafnium	—	—	—
Holmium	—	—	—
Indium	Internal standard	Internal standard	Internal standard
Iodine	1.2	0.4	0.05
Iridium	—	—	—
Iron	6.9	4.0	0.99
Lanthanum	—	—	—
Lead	66	82	7.1
Lithium	7.3	9.8	0.25
Lutecium	—	—	—
Magnesium	8.7	3.8	1.3
Manganese	2.1	1.2	0.07
Mercury	0.46	0.40	0.028
Molybdenum	5.7	1.7	0.35
Neodymium	—	—	—
Nickel	1.4	0.08	0.02
Niobium	0.2	0.1	<0.02
Nitrogen	NR	NR	NR
Osmium	—	—	—
Oxygen	NR	NR	NR
Palladium	—	—	—
Phosphorus	61	9.8	0.48
Platinum	—	—	—
Potassium	44500	2500	64
Praseodymium	—	—	—
Rhenium	Internal standard	Internal standard	Internal standard
Rhodium	—	—	—
Rubidium	12	3.5	0.87
Ruthenium	—	—	—
Samarium	—	—	—
Scandium	<0.4	<0.2	<0.05
Selenium	8.0	4.0	<0.3

Table 2 (contd.)

Element	100 % tobacco	50 % Cytrel	100 % Cytrel
Silicon	100	58	14
Silver	2.1	<0.4	0.1
Sodium	910	10800	2500
Strontium	1.8	0.2	0.07
Sulfur	17100	3500	870
Tantalum	—	—	—
Tellurium	<1	<0.7	<0.2
Terbium	—	—	—
Thallium	2.0	1.7	1.0
Thorium	<1	<0.7	<0.2
Thulium	—	—	—
Tin	8.0	2.8	<0.1
Titanium	0.5	0.3	0.2
Tungsten	—	—	—
Uranium	<0.4	<0.2	<0.05
Vanadium	4.3	0.5	0.02
Ytterbium	—	—	—
Yttrium	0.4	<0.1	1.1
Zinc	250	69	3.7
Zirconium	0.4	0.2	0.01

Notes: Elemental concentration is in ng/cigarette. Those elements which do not have a value stated are < 0.1.

* Mercury determined by combustion/amalgamation atomic fluorescence spectrophotometry.

** NR = Not reported.

amounts by the Cytrel cigarettes. However, the greater glycerol delivery was expected because [1] glycerol has been included in the Cytrel formulation and [2] the control tobacco contained no glycerol or other humectant.

Within the precision of the analysis ($\pm 50\%$ relative) all elements measured with the possible exception of sodium were delivered in lower amounts by Cytrel than by tobacco cigarettes (Table 2). The possibility of contamination of the samples by sodium compounds cannot be dismissed from the consideration of the sodium deliveries. Therefore, it is uncertain whether the sodium deliveries from the 100 % Cytrel and Cytrel-tobacco cigarettes are greater than from the control tobacco cigarette.

The pH of smoke from 100 % Cytrel cigarettes was higher than the pH of smoke from 100 % tobacco cigarettes due in part to the fact that Cytrel deliveries of ammonia were higher than those from flue-cured tobacco (1).

Comparison of Cigarettes Containing Blends of Cytrel and Tobacco

The effects on delivery of representative particulate phase components resulting from the blending of Cytrel with tobacco are shown graphically in Figures 7–8. It is apparent that the delivery of any component is an approximately linear function of blend level. This linearity is an indication that no synergistic effects result from the blending of Cytrel with tobacco. Thus, if the delivery of a component from 100 % Cytrel and from 100 % tobacco is known, the delivery of that

Figure 7. Comparison of component deliveries as a function of Cytrel-tobacco blend levels.

Figure 8. Comparison of component deliveries as a function of Cytrel-tobacco blend levels.

component from a blend of Cytrel and tobacco can be calculated with reasonable accuracy (1).

The results from the determination of nitrosamines and from the elemental survey have not been graphed because of the high uncertainties in these determinations ($\pm 100\%$ relative for nitrosamines and $\pm 50\%$ relative for elements).

SUMMARY

The particulate phases delivered from cigarettes containing 100% Cytrel, 100% flue-cured tobacco, and blends of Cytrel and tobacco have been extensively characterized. Approximately 70% of Cambridge particulate matter (CPM) from 100% Cytrel cigarettes has been defined. A considerably smaller fraction of CPM from 100% tobacco cigarettes has been similarly defined because of its greater complexity.

Only two of the components observed in the particulate phase, glycerol and possibly sodium, were delivered in higher amounts by 100% Cytrel cigarettes than by 100% tobacco cigarettes. For all other species deliveries were lower from 100% Cytrel cigarettes. In cigarettes containing blends of Cytrel and tobacco the deliveries of all components were shown to follow an approximately linear relationship with blend level.

ZUSAMMENFASSUNG

Die Partikelphase des Rauchs von Zigaretten, die jeweils zu 100% aus Cytrel oder „flue-cured“-Tabak und aus Mischungen von Cytrel und Tabak bestanden, wurde eingehend untersucht. Bei den Cytrel-Zigaretten wurde etwa 70% der im Cambridge-Filter zurückgehaltenen Partikelphase (CPM) näher bestimmt. Die Zusammensetzung der Partikelphase der zu 100% aus Tabak bestehenden Zigaretten konnte wegen ihrer größeren Komplexität nur zu einem wesentlich kleineren Teil bestimmt werden.

Nur zwei der in der Partikelphase beobachteten Inhaltsstoffe, Glycerin und möglicherweise Natrium, bildeten sich in den Cytrel-Zigaretten (100%) in größerer Menge als in den zu 100% aus Tabak bestehenden Zigaretten. Die Ausbeute aller anderen Bestandteile war bei den zu 100% aus Cytrel bestehenden Zigaretten geringer. Bei den Zigaretten, die Mischungen aus Cytrel und Tabak enthielten, konnte gezeigt werden, daß die Ausbeute aller Inhaltsstoffe der Partikelphase in ungefähr linearem Verhältnis zu den Mischungsanteilen stand.

RESUME

Les phases particulières produites par des cigarettes contenant 100% de tabac, 100% de Cytrel, et divers mélanges de tabac et de Cytrel ont été analysées de façon détaillée. Environ 70% de la matière particulaire Cambridge (CPM) ont été définis pour les cigarettes de Cytrel pur. Pour les cigarettes de tabac pur, on n'a pu définir de la même manière qu'une fraction beaucoup moins importante de la matière particulaire Cambridge vu sa complexité.

De tous les composés observés dans la phase particulaire, deux seulement ont été produits en quantités plus importantes par les cigarettes 100% Cytrel que par les cigarettes de tabac pur: il s'agit du glycérol, et peut-être du sodium. Tous les autres composés ont été produits en moindre quantité par les cigarettes 100% Cytrel. Pour les cigarettes contenant du Cytrel et du tabac, les productions semblent suivre une relation à peu près linéaire par rapport aux niveaux de mélange.

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