

Chemical Studies on Tobacco Smoke

XX: Smoke Analysis of Cigarettes Made from Bright Tobaccos Differing in Variety and Stalk Positions*

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It has been demonstrated that nicotine, carbon monoxide, hydrogen cyanide, volatile phenols, polynuclear aromatic hydrocarbons (PAH), and other compounds contribute to the toxicity and/or carcinogenicity of tobacco smoke (1, 2). Previous studies have shown that leaves from different stalk positions vary in physical and chemical properties and thus affect the smoke composition of cured tobacco (3, 4). For example, the percentages of nicotine, petroleum-ether extractables, and water-soluble acids are greater in leaves taken from the top of the plant, whereas the reverse is true for cellulose and alkali nitrate.

Based on these observations, it is logical to assume that the concentrations of many toxic or carcinogenic substances in tobacco smoke vary with the stalk position of the leaf for a given tobacco type (5-7). Detailed chemical-analytical data are needed to establish correlations between specific precursors in the tobacco leaf, the stalk position of the leaf, and the smoke concentration of toxic compounds in the smoke for each of the four major tobacco types: Bright, Burley, Maryland and Turkish. The present study is a report on such correlations for bright tobacco varieties.

MATERIALS AND METHODS

Apparatus

Cigarettes were smoked with a 20-channel Phipps and Bird automatic machine (8) for the analyses of total pH, hydrogen cyanide, acetaldehyde, acrolein, volatile phenols, total particulate matter (TPM), and nicotine, with an H. Borgwaldt piston-type smoking machine for single cigarettes for carbon monoxide and carbon dioxide, and

with a 20-channel automatic smoker RM 20/68 (9) for the analyses of the polynuclear aromatic hydrocarbons (PAH), benz(a)anthracene (BaA), and benzo(a)pyrene (BaP). The cigarettes were smoked in a humidity-controlled room with a relative humidity of $60 \pm 5\%$ and at temperatures of $22 \pm 2^\circ \text{C}$.

A Perkin-Elmer gas chromatograph Model 154D with thermo-conductivity detector was used for the smoke analysis of acetaldehyde, acrolein, and water, a Hewlett-Packard 5700A gas chromatograph with a T.C.-detector with a 1 mV recorder for CO and CO₂, and a Perkin-Elmer Modell 800 with dual-flame ionization detector for volatile phenols and nicotine. The β -radiation was counted with a Nuclear-Chicago Scintillation System 720. Ultraviolet-absorbance measurements were obtained with a Cary Model 11 recording spectrophotometer. Evaporations were carried out under reduced pressure with water-bath temperatures below 45°C . The laboratories were illuminated with yellow light (Sylvania Electric Tubes F-40 G.O.), which excludes radiation below 450 m μ .

Reagents

All organic solvents were spectrograde, other chemicals were analytical reagent grade. Woelm neutral alumina (activity II) was purchased from Waters Associates, SE-30, Carbowax 550, Gas Chrom P and Q, and Poropak Q5 from Applied Science Laboratories. The reference compounds were purified by column chromatography, and their purity was established by gas chromatography.

Internal Standards

Phenol-U-¹⁴C (25.9 mCi/mM, Amersham/Searle), BaA-9-¹⁴C (5.5 mCi/mM, Amersham/Searle), and BaP-5-¹⁴C (11.3 mCi/mM, Mallinckrodt) were purified by column chromatography on GLPC. Toluene solutions with 0.4% PPO and 0.005% POPOP as scintillators gave efficiencies of 72-74% for the unquenched ¹⁴C-labeled internal standards.

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Tobacco Samples

Bright tobaccos selected for this study differ widely in their total alkaloid levels. Three flue-cured varieties, SC-58, NC-95, Coker-139, and a low nicotine-breeding line, LN-38, were used. Their respective average alkaloid levels were 3.55, 2.74, 1.24, and 0.24% of the dry cured weight. These tobaccos were produced by Dr. J. F. Chaplin at Kinston, N.C., with normal field management and curing practices. Eight hundred plants of each variety were produced, 200 in each of 4 replications. Cured leaves were arbitrarily divided by 8 stalk positions, which were given ascending numbers from bottom to top of each plant. Thirty-two samples, each with 4 replications, or a total of 128 samples of cured leaf, were analyzed for leaf characteristics.

Cigarette Preparation

For cigarette manufacture, leaves from the 4 replications within each of the 32 samples were combined. The tobaccos were stemmed, well mixed, and cut at 30 cuts/inch. Liquid sugar and glycerine were used for casing. All cigarettes produced were 85-mm nonfilter type.

Cigarette Selection

Two hundred cigarettes were placed in a humidity chamber containing a glass vessel filled with glycerol-water and maintained at 22° C ($\pm 2^\circ$). After 24 hours, gas chromatography determined the cigarettes' moisture content to be between 10.8–12.0%. Only those cigarettes weighing within ± 20 mg of the average for 200 cigarettes were used for pressure-drop, static-burning rate, TPM, nicotine, and volatile-phenols determinations. For CO and CO₂ determinations only those average-weight cigarettes were chosen that had a draw resistance within $\pm 7\%$ of the average.

Pressure Drop

The cigarette was placed in a Cambridge filter assembly connected via Teflon cuffs to a type-W Meriam pressure-drop meter with a 20-inch range. The meter was in turn connected to a precision-bore flow-rater tube. A flow rate of 17.5 ml per minute was chosen. Ten determinations were made for each cigarette, and the average value was calculated.

Static-Burning Rate

After the first puff was taken by the smoking machine, the cigarettes were mounted horizontally on a nail. The static-burning rate is equivalent to the time required for a cigarette to burn from 10 mm to 62 mm without being puffed. Ten determinations were made for each cigarette, and the average value was calculated.

pH of Mainstream Smoke

pH was measured using a Beckman Combination Electrode No. 39,183, modified according to Sensabaugh and Cundiff (10), and connected to a Beckman Ex-

pandomatic SS-2 pH meter with a 100 mV recorder. The pH meter was calibrated from pH 4.5 to 9.0 with buffer solutions. For three analyses the average of the minimum, the mean, and the maximum of the pH for each puff could be determined within ± 0.1 . The first two puffs were disregarded because they are affected by igniting and other factors.

Carbon Monoxide and Carbon Dioxide

Conditioned cigarettes were selected by weight and pressure drop and smoked through a Cambridge filter attached to a Borgwaldt single smoker (8). The gas phase was blown into a modified one-l hospital bag with inlet tube, and the bag was subsequently transferred to the cigarette holder. A 35-ml aliquot was taken, using a piston-type syringe, and blown into the sampling valve. A 0.5-ml sample was taken from the latter and injected into the gas chromatograph. A 6-mm \times 4-m stainless-steel column was used for the separation, first with 1.5 m of silica gel (Fisher S-157), then with 0.3 m of powdered iodine pentoxide, 0.05 m of silver powder, and, finally, with 2.15 m of silica gel. Helium served as carrier gas (80 ml/min.). The oven temperature was 125° C, the detector temperature 250° C. The T.C. current was 225 mA. The retention time for CO₂ was 3 min., for CO 6 min. Nitrogen containing 1.02% CO, 5.01% CO₂, and 5.07% CO and 10.2% CO₂ was used as a standard. For the actual analysis, each cigarette was smoked twice, and three samples were taken from the gas phase of each cigarette.

Hydrogen Cyanide

HCN was determined, using a slight modification of the method developed by Artho and Koch (11). For each determination, the mainstream smoke from one cigarette smoked under standard conditions was passed through a Cambridge filter into a 250-ml gas-wash bottle containing 0.1 N NaOH solution. HCN trapped on the Cambridge filter was then extracted from the glass fiber into a volumetric flask by filtration with two 35-ml portions of 0.1 N NaOH. The filtered extract was brought to a volume of 100 ml by the further addition of NaOH. A 2-ml aliquot was transferred into a 250-ml separatory funnel, and the following compounds were added: 50 ml of water, 3 ml of Br₂-solution (5 ml Br₂ in 1 liter of 2 N HCl), 3 ml Na₃AsO₃-solution (26 g As₂O₃, 23 g NaOH), 700 ml of H₂O (adjusted to pH 4–5 with 0.1 N HCl and diluted to 1 liter), 20 ml iso-amylalcohol, 5 ml benzidine-pyridine solution (10 ml of a solution of 2 g benzidine-hydrochloride), 0.2 ml conc. HCl in 100 ml H₂O, and 100 ml of a solution containing 25 ml pyridine and 2 ml conc. HCl in 75 ml H₂O. The mixture was shaken for 1 min. and allowed to stand for 30 min. The iso-amylalcohol layer was spectrophotometrically examined at 480 nm. A solution containing 1 mg/ml was used as a standard.

The amount of HCN in the gas phase was determined in the same way. Each determination for both the gas and particulate phases was run in triplicate.

Acetaldehyde and Acrolein

One cigarette was smoked under standard conditions into a specially designed trap with a free volume of about 60 ml (1). After between 7–15 puffs were taken, leaving a 23-mm butt, the mainstream smoke was collected in a trap immersed in a dry ice/acetone bath. The trap was connected to a modified Perkin-Elmer Model 154D gas chromatograph by using it as a loop for the sampling valve.

A stream splitter with the ratio of 1:24 was built into the gas chromatograph. Preheated helium at a flow rate of 40 ml/min. was used as carrier-flow gas. The trap was brought to room temperature and then kept in a water bath at 30° C. After 5 min. 4% of the trapped gas phase was introduced into the column. The column conditions were as follows: 3-mm \times 6.6-m column of 15% Carbowax 550 on Gas Chrom P, column temperature 60° C isothermal. The observed retention time for acetaldehyde was 5.3 ± 0.3 min., and for acrolein 10.5 ± 0.5 min. Each value is the average of three analyses.

Volatile Phenols

Ten cigarettes were smoked under standard conditions. The mainstream smoke was passed through a Cambridge filter into a 250-ml gas-wash bottle filled with 25 ml of a 5% NaOH solution. Phenol- $U-^{14}C$, added to the NaOH solution, functioned as a control. The saturated Cambridge filter, the trapping solution, and the rinsings were transferred into a 500-ml distillation flask, acidified with 2N H_2SO_4 , and steam-distilled. The distillate was collected in a flask, which contained a few ml of 5% NaOH solution. After 500 ml of condensate were collected, the basic solution was twice extracted with 120 ml of ether. The lower layer was cooled, acidified, and twice extracted with 120 ml ether. The combined ether extracts were twice extracted with 100 ml of saturated $NaHCO_3$ solution. The dried ether solution was then filtered through a cotton plug, and the retained Na_2SO_4 was washed with ether.

The combined ether layers were concentrated to between 5–10 ml by evaporation through a distilling column. This remaining solution was transferred to a 20-ml flask, and the column was rinsed with 1 ml n-hexane. The solution was then further concentrated to 1.0 ml. Aliquots were analyzed by gas chromatography on a 1.7-m-long column of 5% SE-30 on 80/100 mesh Gas Chrom Q, using a flame ionization detector. Helium, heated to 100° C and at a flow rate of 40 ml/min., was used as the carrier gas. The retention times were as follows:

phenol:	2.95 min.
o-cresol:	4.3 min.
m- and p-cresol:	4.9 min.
2,4- and 2,5-dimethylphenol:	7.5 min.
m- and p-ethylphenol:	8.5 min.

Fifty μ l were diluted with scintillator solution (toluene containing 0.4% PPO and 0.005% POPOP) and counted

for β -activity. In general, 85–95% of the added ^{14}C -labeled phenol was recovered in the final concentration. The results of the analysis are the average values from two runs.

Total Particulate Matter (TPM)

Cigarettes were smoked under standard conditions through a Cambridge filter pad CM-113 (8). The wet particulate matter from one cigarette was determined as the average from 4 \times 4 cigarettes. For the moisture determination, the Cambridge filter packed with wet particulate matter was placed in a reagent vial with 2-propanol (10 ml), covered with a rubber stopper, and shaken mechanically for 2 hrs. Three 10- μ l aliquots of the isopropanol extract were analyzed by gas chromatography, using a 2-m column filled with 80/100-mesh Poropak QS in conjunction with a thermal-conductivity detector. The injector-block temperature was 220° C, the column temperature 190° C, and the helium gas-flow rate was 40 ml/min. The retention times for water and 2-propanol were 4.0 and 5.2 min., respectively. The blank was a 10-ml 2-propanol extract of an unused Cambridge filter. The final report lists the average values from two different sets of Cambridge filters, loaded with the TPM from 4 cigarettes each. The experimental deviation was less than $\pm 5\%$.

Nicotine

A Cambridge filter packed with the TPM of four cigarettes was placed in a glass vial with 10 ml methanol and shaken mechanically for 2 hrs. Three 10- μ l aliquots of the methanol extract were analyzed by gas chromatography on a 4-m column, filled with 10% SE-30 on 60/80 mesh Gas Chrom P, in conjunction with a flame ionization detector. The injection-block temperature was 220° C, and the column temperature was 190° C. The average retention times for nicotine and nornicotine were 8.0 and 10.0 min., respectively. The blanks were methanol extracts of unused Cambridge filters. The nicotine values represent an average of two runs of Cambridge filters loaded with the TPM from four cigarettes. The experimental deviation was less than $\pm 5\%$.

Benz(a)anthracene [BaA] and Benzo(a)pyrene [BaP]

Three hundred cigarettes were smoked individually with an RM 20/68 automatic smoker under standard conditions. The mainstream smoke passed through 2 cold traps immersed in dry ice/acetone and finally through a Cambridge filter. The latter was changed each time 50 cigarettes had been smoked. The condensates from the traps, the rinsings, and the acetone extracts of the Cambridge filters were combined. Control solutions of BaA- $5-^{14}C$ and BaP- $9-^{14}C$ were added, and the suspension was evaporated to dryness under a reduced pressure of 12 mg Hg at a water-bath temperature of 45° C \pm 5°. The residue was distributed between two pairs of pre-equilibrated solvents: methanol/water(4:1)-

cyclohexane, and cyclohexane-nitromethane. The partition steps concentrated the aromatic nonvolatile hydrocarbons about 10 times and removed the hydrophilic nonvolatile smoke compounds (distribution step I) and the aliphatic hydrocarbons (step II). The latter diminished the ability of the adsorbent in the subsequent column chromatography to separate the PAH from the olefines, terpenes, and certain esters. The residue of the second distribution step was dried in a vacuum desiccator over calcium chloride.

The dry residue (0.5–1.5 g, depending on the type of cigarette analyzed) was dissolved in 2–4 ml n-hexane/benzene (10:1) and chromatographed with n-hexane/benzene (8:1) on 350 g neutral Woelm alumina, activity II. The eluate was collected in 50-ml fractions. Fractions 16–22 and 25–32, which contained the radioactivity, were separately combined and evaporated. The residues (8–30 mg) were applied to 4 sheets of acetylated No. 1 Whatman paper [prep. of acetylated paper (1)], and the paper was developed overnight in a 4:4:1 methanol/ether/water solution.

The BaA and BaP bands were easily located by their fluorescence under ultraviolet light (365 mμ) and R_f of references, average R_f values 0.42 and 0.18. The BaA and BaP bands were cut out and placed in microsoxhlets, and the fluorescent material was eluted with benzene/alcohol (4:1) in a nitrogen atmosphere. The solvent was evaporated on completion, and the residues were dissolved in n-hexane/benzene (4:1) and filtered through small columns of alumina (activity III). The filtrates were evaporated, the residues were dissolved in 10 ml spectrograde cyclohexane, and aliquots were taken for measurement of the ultraviolet spectra and for counting the radioactivity. Approx. 80.0 to 90.0% of the BaA and BaP was recovered.

RESULTS AND DISCUSSION

The mainstream smoke of 32 experimental cigarettes was analyzed. The following chemical indicators, together with their respective parameters, were chosen: combustibility – burning rate; overall toxicity – TPM and nicotine; degree of nicotine toxicity – pH; gas-phase toxicity – CO_2 , CO, and HCN; cilia toxicity – acrolein and acetaldehyde; volatile tumor promoters – phenols; and nonvolatile tumor initiators – BaA and BaP.

As summarized in Tables 1 through 6, the following parameters decrease with increasing height of stalk position of the bright-tobacco leaf:

1. Filling power and pressure drop (Table 1),
2. Combustibility and static-burning rate (Table 1).

These two factors and the change in the chemical composition of the leaves result in increased yields of the following smoke constituents with increase in height of stalk position:

1. TPM (Table 1),
2. Nicotine (Table 1),
3. pH of total smoke (Table 2),

4. Hydrogen cyanide (Table 4),
5. Volatile phenols (Table 5),
6. PAH, as determined with BaA and BaP (Table 6).

Since the combustibility of the tobacco leaf decreases with increased height of stalk position, it is not surprising that the TPM yield increases toward the upper leaves (Table 1). This result agrees with the fact that a relatively high wax content occurs in the upper leaves. TPM is formed in many ways, primarily by distillation, cracking, and pyrosynthesis. Volatile phenols and PAH, however, are formed by free-radical reactions (1, 12). This difference partly explains why the volatile phenols and PAH do not increase to the same extent as TPM (Tables 1, 5 and 6).

The acute toxicity of the particulate matter of tobacco smoke is primarily a function of its pH and the concentration of nicotine. Since both smoke parameters increase with increasing height of position of the bright-tobacco leaf (Tables 1 and 2), one predicts a significantly higher toxicity for the "tar" obtained from the upper leaves than for that from the lower leaves. The increase in nicotine and decrease in acidity of the smoke may be interrelated to each other. This can be read from Tables 1 and 2. Additional studies may confirm the consistency of such a relationship.

Hydrogen cyanide in tobacco smoke derives primarily from the protein portion of the leaf (13). Since the latter increases in relative concentration in the leaf with the increasing height of stalk position, it is not surprising that the upper leaves deliver significantly larger amounts of HCN than the lower leaves (Table 4).

In general, the highest CO measurement, as percent by volume, was made using the smoke of tobacco composed of the middle leaves (Table 3). Carbon monoxide concentration was significantly lower in the smoke of tobacco composed of the leaves from the other stalk positions. The data did not reveal any significant correlation between percent by volume CO_2 in the smoke and stalk position of the leaves composing the tobacco (Table 3). Despite repeated analyses, we found highly elevated values for CO and CO_2 in the smoke of cigarettes No. 24 and 34. It was possible that during the processing of these two tobaccos and/or the preparation of the cigarettes, alterations had occurred that led to these unexpectedly high results.

Acetaldehyde and acrolein are formed in the highest amounts from the middle leaves (Table 4). At present, our data do not offer an explanation for this finding.

The chemical-analytical data presented here indicate that the relative potential toxicity and tumorigenicity of the smoke from bright tobacco increases with increasing height of stalk position of the leaf. This result requires, however, a detailed study of possible correlations between tobacco-leaf compounds and leaf parameters and the toxic agents in the smoke. In the following papers we will discuss our findings on such possible correlations (14).

SUMMARY

Leaves taken from eight different stalk positions of four bright-tobacco varieties were used to make 85-mm cigarettes. The cigarettes were smoked under standard conditions, and the mainstream smoke was analyzed for selected toxic agents. The results demonstrate that the higher the leaf on the stalk, the less its filling power and combustibility and the greater the pH, total particulate matter, nicotine, hydrogen cyanide, volatile phenols, and polynuclear aromatic hydrocarbons of the mainstream smoke. Carbon monoxide, acetaldehyde, and acrolein are found in the highest concentration in the smoke formed from leaves in the middle stalk positions. The concentration of CO₂ was comparable for the smoke from the leaves of all stalk positions of a given bright-tobacco variety.

These chemical-analytical data suggest that the relative potential toxicity and tumorigenicity of the smoke of bright tobacco increase with the ascending stalk position of the leaf.

ZUSAMMENFASSUNG

Cigaretten gleicher Parameter wurden aus vier Virginia-Tabaken acht verschiedener Blattpositionen hergestellt. Die so erhaltenen 32 experimentellen Cigaretten wurden unter Standardbedingungen abgeraucht, und der Hauptstromrauch wurde auf ausgewählte toxische Verbindungen hin analysiert. Die Ergebnisse zeigen, daß mit zunehmender Blatthöhe die Füllfähigkeit und die Brennfähigkeit des Tabaks abnehmen, während die folgenden Hauptstromrauchparameter ansteigen: pH, Gesamtkondensat, Nikotin, Blausäure, flüchtige Phenole und polyzyklische Kohlenwasserstoffe. Kohlenmonoxid, Acetaldehyd und Acrolein wurden in der höchsten Konzentration im Rauch der Blätter mittlerer Position gefunden. Im Rauch der einzelnen Virginia-Tabake wurden keine signifikanten Unterschiede für Kohlendioxid gefunden.

RESUME

On a fabriqué des cigarettes de 85 mm à partir de feuilles de tabac Bright de quatre variétés différentes et de huit positions différentes sur la tige. Les cigarettes ont été fumées dans des conditions standard, et l'on a analysé la teneur en agents toxiques déterminés du flux principal de fumée. Les résultats démontrent que plus la feuille est haute sur la tige, moins sont importants le pouvoir de remplissage et l'aptitude à brûler, et plus sont élevés le pH, la matière particulaire totale, et la teneur en nicotine,

en acide cyanhydrique, en phénols volatiles et en hydrocarbures aromatiques polynucléaires. La plus haute concentration en monoxyde de carbone, acétaldéhyde et acroléine se trouve dans la fumée provenant de feuilles de position milieu de tige. Les concentrations de CO₂ sont équivalentes pour les feuilles de toute position provenant de la même variété de tabac Bright.

Ces données chimiques analytiques suggèrent que la toxicité potentielle relative et la tumogénescence de la fumée des feuilles des tabacs Bright augmentent en fonction de la hauteur des feuilles sur la tige.

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Table 1. Analyses of cigarettes made from eight stalk positions of four flue-cured varieties.

Sample no.	Average wt. of cig. (mg)	Static-burning rate (mg/min.)	Pressure drop (inches)	Average number of puffs/cig.	TPM (mg/cig.)	Nicotine (mg/cig.)	TPM minus nicotine
SC-58:							
11	1,147	69.1	2.93	10.0 (8.7)*	20.90 (18.2)	0.91 (0.79)	19.99 (17.41)
12	929	68.0	2.63	9.0 (9.7)	27.80 (29.9)	1.09 (1.17)	26.71 (28.73)
13	1,137	63.5	3.55	9.9 (8.7)	32.76 (28.8)	2.31 (2.03)	30.45 (26.77)
14	985	59.1	2.40	9.3 (9.4)	39.81 (40.0)	4.40 (4.47)	35.41 (35.53)
15	1,050	59.0	2.57	9.3 (8.9)	40.45 (38.5)	4.80 (4.57)	35.65 (33.93)
16	1,026	57.5	1.95	9.8 (9.6)	44.43 (43.3)	5.60 (5.46)	38.83 (37.84)
17	1,137	55.3	2.10	10.9 (9.6)	46.44 (40.8)	6.65 (5.85)	39.79 (34.95)
18	1,200	56.5	1.92	11.4 (9.5)	53.78 (44.8)	8.04 (6.70)	45.74 (38.10)
LN-38:							
21	1,155	64.5	3.32	9.4 (8.1)	19.70 (17.06)	0.22 (0.19)	19.48 (16.87)
22	1,063	64.7	7.22	9.0 (8.5)	15.12 (14.22)	0.12 (0.11)	15.03 (14.11)
23	1,033	66.7	3.27	8.6 (8.3)	27.62 (26.74)	0.28 (0.27)	27.34 (26.47)
24	839	66.6	1.83	7.0 (8.3)	28.00 (33.37)	0.32 (0.38)	27.68 (32.99)
25	955	56.8	2.15	8.3 (8.7)	31.43 (32.91)	0.30 (0.31)	31.13 (32.60)
26	1,067	56.4	2.35	8.9 (8.3)	37.36 (35.01)	0.93 (0.87)	36.43 (34.14)
27	1,072	60.6	1.93	9.0 (8.4)	32.50 (30.32)	0.30 (0.28)	32.20 (30.04)
28	1,062	51.0	1.90	9.7 (9.1)	35.20 (33.15)	0.49 (0.46)	34.71 (32.69)
Coker-139:							
31	1,000	68.9	4.43	8.0 (8.0)*	17.26 (17.26)	0.43 (0.42)	16.84 (16.84)
32	975	61.3	7.80	8.2 (8.4)	16.56 (16.99)	0.43 (0.44)	16.13 (16.55)
33	1,010	66.4	6.10	8.9 (8.8)	15.40 (15.25)	0.56 (0.55)	14.84 (14.70)
34	858	61.4	3.30	7.8 (9.1)	27.46 (32.01)	1.08 (1.26)	26.38 (30.75)
35	980	66.4	3.58	8.28(8.5)	32.90 (33.57)	1.86 (1.90)	30.04 (31.67)
36	1,050	60.9	3.23	9.2 (8.8)	36.40 (34.67)	2.76 (2.63)	33.64 (32.04)
37	1,000	55.3	2.53	9.2 (9.2)	40.15 (40.15)	3.12 (3.12)	37.03 (37.03)
38	1,065	58.3	2.00	10.0 (9.4)	46.18 (43.36)	3.17 (2.98)	43.01 (40.38)
NC-95:							
41	1,000	59.9	6.4	8.2 (8.2)	17.18 (17.18)	0.77 (0.77)	16.41 (16.41)
42	930	64.0	5.45	7.9 (8.5)	23.33 (25.09)	0.99 (1.06)	22.34 (24.03)
43	900	55.7	3.43	8.1 (9.0)	30.0 (33.33)	2.24 (2.49)	27.76 (30.84)
44	995	58.5	3.9	8.8 (8.8)	34.0 (34.17)	3.18 (3.20)	30.82 (30.97)
45	1,075	54.1	3.9	9.9 (9.2)	38.85 (36.14)	3.63 (3.38)	35.22 (32.79)
46	1,110	51.0	2.38	9.9 (8.9)	41.70 (37.68)	4.54 (4.09)	37.16 (33.59)
47	1,250	50.7	3.58	12.9 (10.3)	45.58 (36.46)	4.38 (3.50)	41.20 (32.96)
48	1,390	47.7	3.2	15.6 (11.2)	48.94 (35.21)	4.93 (3.55)	44.01 (31.66)

* The numbers in parentheses refer to those one would obtain if the weight of the cigarettes were 1,000 mg.

Table 2. pH of the mainstream smoke of cigarettes made from eight stalk positions of four flue-cured varieties*.

Sample no.	3rd-puff pH			5th-puff pH			7th-puff pH			Last-puff pH			
	Low	High	Mean	Low	High	Mean	Low	High	Mean	Low	High	Mean	
SC-58:													
11	5.96	6.22	6.09	6.02	6.26	6.14	6.04	6.24	6.14	(9)**	6.01	6.15	6.08
										(10)	5.98	6.11	6.05
12	6.06	6.31	6.19	6.12	6.31	6.22	6.11	6.25	6.18	(9)	6.00	6.13	6.07
13	6.27	6.55	6.41	6.33	6.58	6.46	6.35	6.57	6.46	(9)	6.34	6.48	6.41
										(10)	6.32	6.43	6.38
14	6.22	6.54	6.38	6.47	6.69	6.58	6.53	6.67	6.60	(9)	6.54	6.67	6.61
15	6.42	6.62	6.52	6.54	6.69	6.62	6.59	6.68	6.64	(9)	6.62	6.72	6.67
16	6.55	6.85	6.70	6.74	6.94	6.84	6.82	7.02	6.92	(9)	6.88	7.18	6.98
										(10)	6.93	7.11	7.02
17	6.72	6.90	6.82	6.88	7.05	6.97	6.94	7.10	7.02	(9)	6.99	7.14	7.07
										(10)	7.02	7.18	7.10
18	6.89	7.04	6.97	6.91	7.10	7.01	6.93	7.10	7.02	(9)	6.98	7.17	7.08
										(11)	7.02	7.24	7.14
LN-38:													
21	5.93	6.14	6.04	5.93	6.13	6.03	5.87	6.06	5.97	(9)	5.80	5.96	5.88
22	5.92	6.06	5.99	5.90	6.05	5.98	5.85	6.01	5.93	(9)	5.78	5.89	5.84
23	5.71	5.88	5.80	5.77	5.90	5.84	5.76	5.86	5.81	(9)	5.60	5.75	5.70
24	5.85	6.09	5.97	5.86	6.04	5.95	5.72	5.90	5.81	(7)	5.72	5.90	5.81
25	5.66	5.91	5.79	5.78	5.95	5.87	5.78	5.90	5.84	(8)	5.78	5.87	5.83
26	5.93	6.20	6.07	6.01	6.28	6.15	6.05	6.23	6.14	(9)	6.02	6.12	6.07
27	5.90	6.22	6.06	5.92	6.15	6.04	5.89	6.07	5.98	(9)	5.82	5.95	5.89
28	5.93	6.22	6.08	6.00	6.22	6.11	6.01	6.16	6.09	(9)	5.93	6.06	6.00
										(10)	5.85	6.00	5.93
Coker-139:													
31	6.18	6.36	6.27	6.16	6.33	6.25	6.14	6.27	6.21	(8)	6.05	6.16	6.11
32	6.03	6.22	6.13	6.07	6.26	6.17	6.07	6.21	6.14	(8)	5.99	6.11	6.05
33	5.89	6.05	5.97	5.94	6.09	6.02	5.96	6.11	6.04	(9)	5.91	6.02	5.97
34	5.95	6.12	6.04	5.97	6.12	6.05	5.92	6.04	5.98	(8)	5.85	5.97	5.91
35	5.87	6.03	5.95	5.92	6.04	5.98	5.92	6.01	5.97	(8)	5.88	5.95	5.92
36	5.95	6.10	6.03	6.05	6.19	6.12	6.09	6.19	6.14	(9)	6.09	6.14	6.12
37	6.01	6.18	6.10	6.09	6.24	6.17	6.14	6.26	6.20	(9)	6.21	6.37	6.29
38	6.12	6.40	6.26	6.20	6.46	6.33	6.23	6.49	6.36	(9)	6.29	6.48	6.39
										(10)	6.29	6.45	6.37
NC-95:													
41	6.02	6.16	6.09	6.06	6.19	6.13	6.07	6.17	6.12	(8)	6.03	6.12	6.08
42	5.97	6.09	6.03	6.07	6.16	6.12	6.08	6.16	6.12	(8)	6.04	6.11	6.08
43	6.11	6.25	6.18	6.19	6.30	6.25	6.23	6.30	6.27	(8)	6.23	6.30	6.27
44	5.90	6.00	5.95	5.98	6.09	6.04	6.03	6.09	6.08	(9)	6.07	6.13	6.10
45	6.24	6.42	6.33	6.39	6.53	6.46	6.45	6.55	6.50	(9)	6.48	6.57	6.53
										(10)	6.50	6.59	6.55
46	6.28	6.58	6.43	6.41	6.68	6.55	6.52	6.73	6.63	(9)	6.57	6.71	6.64
										(10)	6.58	6.73	6.66
47	6.38	6.67	6.53	6.54	6.87	6.71	6.69	6.87	6.78	(9)	6.73	6.92	6.83
										(11)	6.78	6.95	6.87
										(13)	6.83	6.99	6.91
48	6.46	6.79	6.63	6.57	6.83	6.70	6.62	6.83	6.73	(9)	6.64	6.85	6.75
										(11)	6.68	6.92	6.80
										(13)	6.74	6.93	6.84
										(15)	6.78	6.97	6.88

* Values are averages of 3 tests.

** Number in parentheses is number of puffs.

Table 3. Carbon monoxide (CO) and carbon dioxide (CO₂) in cigarette mainstream smoke from tobaccos from selected varieties and stalk positions.

Sample no.	CO		CO ₂		CO/CO ₂ ratio
	(vol. %)	(mg/cig.)	(vol. %)	(mg/cig.)	
SC-58:					
11	3.87	15.5 (13.5)*	9.53	60.0 (52.3)*	3.87
12	4.37	16.6 (17.0)	10.27	61.6 (63.2)	3.71
13	4.31	17.3 (15.2)	10.75	67.8 (59.6)	3.91
14	5.24	19.5 (19.8)	10.74	63.0 (64.0)	3.23
15	5.37	20.5 (19.5)	10.63	63.8 (60.8)	3.11
16	5.36	21.5 (21.0)	10.17	64.1 (62.5)	2.98
17	5.24	23.1 (20.3)	9.53	66.1 (58.1)	2.88
17 (repeat)	5.35	23.6 (20.8)	9.93	68.9 (60.6)	2.90
18	4.93	22.9 (19.1)	9.70	71.1 (59.3)	3.10
LN-38:					
21	4.27	18.0 (15.9)	10.12	66.9 (57.9)	3.71
22	4.18	17.3 (16.3)	10.06	63.4 (59.6)	3.67
23	4.43	17.7 (17.1)	10.14	63.9 (61.9)	3.61
24	6.62	18.6 (21.5)**	11.45	50.5 (58.2)	2.71
24 (repeat a)	6.37	19.5 (22.5)**	11.83	51.5 (59.4)	2.64
24 (repeat b)	6.51	19.9 (23.0)**	11.61	51.2 (59.1)	2.57
25	4.67	16.8 (17.6)	9.77	55.4 (58.0)	3.27
26	4.73	20.9 (19.6)	10.03	69.5 (65.1)	3.32
27	4.84	20.7 (19.3)	9.94	64.7 (60.4)	3.12
28	4.35	19.2 (18.1)	9.24	64.1 (60.4)	3.33
Cocker-139:					
31	3.44	13.0 (13.0)	8.95	53.4 (53.4)	4.10
32	3.75	14.2 (14.6)	9.24	55.1 (56.5)	3.88
33	4.07	13.8 (13.7)	9.32	49.8 (49.3)	3.61
34	5.31	15.9 (18.5)	10.05	47.4 (55.2)	2.98
34 (repeat)	5.46	15.3 (17.8)	10.77	50.8 (59.2)	3.32
35	4.46	15.2 (15.5)	9.61	51.3 (52.3)	3.37
36	4.62	18.5 (17.6)	10.06	63.4 (60.4)	3.42
37	4.29	17.2 (17.2)	9.21	58.0 (58.0)	3.37
38	4.24	18.7 (17.6)	9.93	68.8 (64.6)	3.68
NC-95:					
41	3.92	12.6 (12.6)*	9.44	47.6 (47.6)*	3.78
42	4.40	15.9 (17.1)	10.09	57.3 (61.6)	3.60
43	4.85	16.6 (18.4)	10.00	53.5 (59.4)	3.22
44	5.26	18.4 (18.5)	10.15	57.6 (57.9)	3.13
45	4.95	21.8 (20.3)	10.03	69.5 (64.7)	3.18
45 (repeat)	5.13	19.5 (18.1)	9.92	60.6 (56.4)	3.11
46	4.43	20.7 (18.6)	9.59	72.5 (65.3)	3.50
47	3.48	19.1 (15.3)	7.90	68.0 (54.4)	3.56
48	3.35	21.5 (15.5)	7.65	77.1 (55.5)	3.59

* The numbers in parentheses refer to those values one would obtain if the weight of the cigarettes were 1,000 mg.

** The weight of the cigarette was 867 mg. In an earlier No. 24 sample, the average weight per cigarette was 839 mg, its CO values were 5.84 vol. % and 18.8 mg/cig. (22.4 mg/lg tobacco), and its CO₂ values were 10.59 vol. % and 53.4 mg/cig. (63.6).

Table 4. Analytical results of cigarettes made from eight stalk positions of four flue-cured varieties.

Sample no.	Acetaldehyde ($\mu\text{g}/\text{cig.}$)	Acrolein ($\mu\text{g}/\text{cig.}$)	Hydrogen cyanide		Total/cig. (μg)
			Gas phase ($\mu\text{g}/\text{cig.}$)	Part. phase ($\mu\text{g}/\text{cig.}$)	
SC-58:					
11	984 (858)*	96 (84)*	227	78	305 (266)*
12	1,070 (1,150)	95 (102)	240	74	314 (338)
13	1,180 (1,040)	98 (86)	304	137	441 (388)
14	1,190 (1,210)	91 (92)	250	227	477 (484)
15	890 (848)	90 (86)	348	196	544 (518)
16	900 (878)	68 (66)	419	255	674 (657)
17	805 (708)	65 (57)	418	263	681 (600)
18	800 (667)	51 (43)	479	203	682 (568)
LN-38:					
21	840 (730)	85 (74)	198	17	215 (186)
22	975 (917)	89 (84)	176	26	202 (190)
23	1,150 (1,110)	89 (86)	271	32	303 (293)
24	950 (1,130)	87 (104)	347	32	379 (452)
25	987 (1,030)	64 (67)	352	59	411 (430)
26	1,060 (993)	70 (66)	350	97	364 (341)
27	1,050 (979)	60 (56)	335	142	477 (445)
28	1,035 (975)	59 (56)	320	161	481 (453)
Coker-139:					
31	865 (865)	65 (65)	140	41	181 (181)
32	1,120 (1,150)	91 (93)	141	40	181 (186)
33	1,250 (1,240)	97 (96)	158	38	196 (194)
34	1,240 (1,450)	97 (113)	188	58	246 (287)
35	1,280 (1,310)	102 (104)	233	68	301 (307)
36	1,125 (1,070)	79 (75)	212	80	292 (278)
37	1,025 (1,025)	78 (78)	277	232	509 (509)
38	1,080 (1,010)	70 (66)	340	259	599 (562)
NC-95:					
41	887 (887)	80 (80)	219	78	297 (297)
42	1,075 (1,160)	88 (95)	249	111	360 (387)
43	1,100 (1,220)	91 (101)	208	123	331 (368)
44	1,120 (1,130)	94 (94)	258	191	449 (451)
45	1,040 (967)	79 (73)	233	178	411 (382)
46	980 (883)	77 (69)	266	199	465 (419)
47	980 (784)	74 (59)	300	255	555 (444)
48	940 (676)	74 (53)	347	237	584 (420)

* The numbers in parentheses refer to those values one would obtain if the weight of the cigarettes were 1,000 mg.

Table 5. Analytical results of cigarettes made from eight stalk positions of four flue-cured varieties.

Sample no.	Phenol (g/cig.)	o-Cresol (g/cig.)	m+p-Cresol (g/cig.)	2,3-+2,5-Dimethylphenol (g/cig.)	p-Ethylphenol (g/cig.)	Total volatile phenols measured (g/cig.)
SC-58:						
11	70.6 (61.55)*	17.3 (15.08)	44.3 (38.62)	6.8 (5.93)	11.6 (10.11)	150.6 (131.3)
12	89.3 (96.12)	18.0 (19.38)	49.1 (52.85)	7.4 (7.97)	12.2 (13.13)	176.0 (189.5)
13	162.0 (142.5)	25.8 (22.69)	61.1 (53.74)	9.6 (8.44)	18.5 (16.27)	277.0 (243.6)
14	191.0 (193.9)	24.9 (25.28)	63.0 (64.00)	9.4 (9.54)	14.8 (15.03)	303.1 (307.8)
15	186.0 (177.1)	23.3 (22.19)	56.7 (54.00)	8.8 (8.38)	16.4 (15.62)	291.2 (277.3)
16	197.0 (192.0)	25.9 (25.24)	65.9 (64.23)	8.3 (8.09)	20.2 (19.69)	317.3 (309.3)
17	275.0 (241.9)	35.3 (31.05)	88.1 (77.48)	11.5 (10.11)	24.3 (21.37)	434.2 (381.9)
18	306.0 (255.0)	35.9 (29.92)	96.2 (80.17)	10.8 (9.00)	22.3 (18.58)	471.2 (392.7)
LN-38:						
21	42.4 (36.71)	9.4 (8.14)	19.6 (16.97)	7.9 (6.84)	11.1 (9.61)	90.4 (78.3)
22	55.7 (52.4)	13.2 (12.42)	29.4 (27.66)	10.7 (10.07)	15.4 (14.49)	124.4 (117.0)
23	81.5 (78.9)	18.6 (18.01)	39.7 (38.43)	14.8 (14.33)	16.5 (15.97)	171.1 (165.6)
24	87.4 (104.17)	20.2 (24.08)	40.5 (48.27)	15.1 (18.00)	17.9 (21.33)	181.1 (215.9)
25	106.0 (111.0)	26.2 (27.43)	58.3 (61.05)	10.4 (10.89)	18.1 (18.95)	219.0 (229.3)
26	130.0 (121.8)	28.8 (26.99)	63.6 (59.61)	12.0 (11.25)	20.6 (19.31)	255.0 (239.0)
27	147.0 (137.1)	35.8 (33.4)	75.8 (70.71)	13.4 (12.5)	24.4 (22.76)	296.4 (276.5)
28	176.0 (165.7)	43.2 (40.68)	90.9 (56.11)	13.0 (12.24)	19.8 (18.64)	342.9 (293.4)
Coker-139:						
31	55.4 (55.4)	10.8 (10.8)	21.4 (21.4)	10.2 (10.2)	16.4 (16.4)	114.2 (114.2)
32	58.9 (60.41)	11.4 (11.69)	23.8 (24.41)	11.4 (11.69)	16.7 (17.13)	122.2 (125.3)
33	99.5 (98.51)	21.5 (21.29)	44.1 (43.66)	12.6 (12.48)	17.8 (17.62)	195.5 (193.6)
34	124.0 (144.5)	24.6 (28.67)	58.7 (68.41)	14.7 (17.13)	20.7 (24.13)	242.7 (282.8)
35	130.0 (132.7)	25.9 (26.43)	56.7 (57.86)	15.2 (15.51)	21.4 (21.84)	249.2 (254.3)
36	128.0 (121.9)	24.7 (23.52)	59.0 (56.19)	15.1 (14.38)	23.5 (22.38)	250.3 (238.4)
37	172.0 (172.0)	31.8 (31.8)	62.4 (62.4)	15.7 (15.7)	24.0 (24.0)	305.9 (305.9)
38	173.0 (162.4)	34.7 (32.58)	66.8 (62.72)	16.5 (15.49)	28.1 (26.38)	491.1 (299.6)
NC-95:						
41	49.4 (49.4)*	13.8 (13.8)	29.6 (29.6)	6.1 (6.1)	10.5 (10.5)	109.4 (109.4)
42	88.2 (94.84)	18.1 (19.46)	42.7 (45.91)	8.8 (9.46)	16.0 (17.2)	173.8 (186.9)
43	109.0 (121.1)	16.4 (18.22)	42.3 (47.00)	6.3 (7.00)	13.2 (14.67)	187.2 (208.0)
44	144.0 (144.7)	24.7 (24.82)	60.8 (61.11)	8.0 (8.04)	16.9 (16.98)	254.4 (255.7)
45	155.0 (144.2)	23.6 (21.95)	62.0 (57.67)	8.4 (7.81)	20.7 (19.26)	269.7 (250.9)
46	235.0 (211.7)	30.4 (27.39)	87.8 (79.10)	9.3 (8.38)	19.6 (17.66)	382.1 (344.2)
47	248.0 (198.4)	32.7 (26.16)	88.9 (71.12)	9.3 (7.44)	26.4 (21.12)	405.3 (324.2)
48	321.0 (230.9)	43.4 (31.22)	122.0 (87.77)	18.3 (13.17)	38.7 (27.84)	543.4 (390.9)

* The numbers in parentheses refer to those values one would obtain if the weight of the cigarettes were 1,000 mg.

Table 6. Analytical results of cigarettes made from eight stalk positions of flue-cured varieties.

Sample no.	BaP (ppm)	BaP (g/100 g tobacco smoked)	BaP (ng/cig.)	BaA (ppm)	BaA (g/100 g tobacco smoked)	BaA (ng/cig.)
SC-58:						
11	0.87	2.17	18.2	1.73	4.31	36.1
12	0.84	3.50	23.3	1.56	6.47	43.1
13	0.69	2.72	22.6	1.32*	5.22*	43.4*
14	0.64	3.52	25.4	1.23*	6.75*	48.7*
15	0.69	3.61	27.8	1.32*	6.93*	53.4*
16	0.61	3.60	27.0	1.17*	6.91*	51.8*
17	0.62	3.45	28.7	1.12	6.27	52.2
18	0.58	3.54	31.1	1.16	7.10	62.4
LN-38:						
21	0.90	2.09	17.7	1.64	3.80	32.2
22	0.85	1.64	12.8	1.61	3.10	24.2
23	0.69	2.50	19.0	1.19*	4.32*	32.8*
24	0.71	3.24	19.9	1.36*	6.22*	38.2*
25	0.73	3.27	22.9	1.40*	6.27*	43.9*
26	0.70	3.34	26.6	1.34*	6.41*	51.0*
27	0.64	2.95	20.8	1.23	5.68	40.0
28	0.66	2.98	23.2	1.21	5.45	42.4
Coker-139:						
31	0.87	2.04	14.9	1.63	3.82	27.9
32	0.85	1.95	14.0	1.58	3.62	26.0
33	0.84	2.00	12.9	1.61*	3.84*	24.8*
34	0.71	2.99	19.4	1.36*	5.74*	37.2*
35	0.70	3.07	23.0	1.34*	5.89*	44.1*
36	0.61	2.89	22.2	1.17*	5.54*	42.6*
37	0.61	3.34	24.4	1.22	6.67	48.7
38	0.63	3.72	29.0	1.21*	7.14*	55.7*
NC-95:						
41	0.96	2.24	16.4	1.84*	4.30*	31.5*
42	0.94	3.21	21.9	2.25	7.70	52.5
43	0.86	3.91	25.8	1.65*	7.50*	49.5*
44	0.71	3.31	24.1	1.36*	6.35*	46.2*
45	0.74	3.67	28.7	1.42*	7.04*	55.1*
46	0.75	3.84	31.2	1.44*	7.37*	59.9*
47	0.75	3.72	34.1	1.44	7.14	65.5
48	0.72	3.49	35.2	1.36	6.60	66.6

BaP — Benzo(a)pyrene

BaA — Benz(a)anthracene

ppm — part per million in dry TPM

* Benz(a)anthracene values were indirectly calculated.