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Introduction

Our understanding of cigarette smoke—its generation, physical composition, toxicity, pharmacology, behavioral effects, and techniques to modify its composition—has advanced considerably since the last review on cigarette smoke in the 1972 report on *The Health Consequences of Smoking.*

Technology has played an important role in advancing our understanding of cigarettes and their resulting smoke. One aspect in particular that has improved our understanding is the development of new instrumentation and miniaturization of analytical tools. For example, Baker (*1*) reported on the use of a fiber-optic probe system for determining and differentiating solid and gas temperatures within the coal of a burning cigarette. The advance made it possible for Osdene (*5*) to define more clearly the reaction mechanisms that occur in the burning cigarette. Such information should make intelligible modification of cigarettes and cigarette smoke more of a science and less of an art. Another example has been the development and refinement of the Thermal Energy Analyzer, which allows scientists to quantify the level of N-nitrosamines in cigarette smoke (*2, 3*). The development of reconstituted tobacco sheet technology, designed, at least in part, for better utilization of the tobacco plant in cigarette manufacture, has given manufacturers additional control over the delivery of certain constituents of cigarette smoke, permitting alteration of the combustion process and consequently the levels of smoke condensate produced (*4*).

In this chapter we will consider the tobacco as a raw material, how it is made into cigarettes, the cigarette smoke generation process, the composition of cigarette smoke, physiological responses to cigarette smoke, the pharmacology of nicotine as a component of cigarette smoke, and efforts to define less hazardous cigarettes through cigarette smoke modification. Also, consideration will be given to the effects of smoke characteristics on smoking behavior and, therefore, on the dose inhaled by man and experimental animals.

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The Cigarette: Composition and Construction

Tobacco, a member of the nightshade family (*28*), is an important agricultural and economic crop that is produced in almost all parts of the world and used in nearly every country. The tobacco plant *Nicotiana tabacum L.* is a native plant of the Americas and is used primarily for the manufacture of cigarettes, cigars, pipe tobaccos, and to a lesser extent for oral consumption. Its dominance for smoking use is generally attributed to a few of its combustion products which induce physiological effects to be discussed later in this chapter. The tobacco plant is an excellent material for research in plant and biological science (*24*).

The characteristics of tobacco smoke are primarily functions of the physical and chemical properties of the leaf; hence, one can approximate the levels of nicotine, tar, and other smoke components based on certain physical and chemical properties of the leaf (*32*). Wide variations in botanical, chemical, and physical characteristics of leaf tobacco are found among the various species, types, varieties, strains, and grades; the quality of the tobacco leaves is predetermined by genetic makeup and subsequently influenced by weather conditions, cultural practices, soil properties, curing, and other post-harvest handling practices (*27*).

The relatively sweet Orinoco-type tobacco, *Nicotiana tabacum L.* was successfully introduced for cultivation in Jamestown, Virginia in 1611 and into Europe, Asia, and South Africa by the early part of the 17th century. Worldwide production has increased in recent years (*26*). During the years 1973 through 1975, worldwide total acreages of tobacco harvested were 10.1, 10.5, and 10.7 million acres; yields per acre were 1,054, 1,080, and 1,088 pounds; and total production was 10.7, 11.4, and 11.7 billion pounds, respectively (*26*).

Asian countries lead the world in tobacco production followed by North America, Europe, and South America (*26*). The highest yield per acre appears to be in the People's Republic of China, followed by the United States. The U.S. production for all types of tobacco in 1975 was 2.19 billion pounds. Table 1 summarizes U.S. tobacco production.

Since 1964, when the first Surgeon General's Report on Smoking and Health was published, there has been a gradual and continued increase in the number of cigarettes manufactured in the United States (*35*). It should be noted, however, that per capita consumption has decreased from 11.53 pounds in 1964 to 9.14 pounds in 1975, and total tobacco consumption has declined from 1.41 billion pounds in 1964 to 1.35 billion pounds in 1975. This reduction is due largely to the reduced waste of the tobacco biomass. These results are described in Figure 1.

Figure 2 describes the tobacco use for men and women 21 and older for the years 1970 and 1975. It should be noted that there was an

TABLE 1.—U.S. tobacco production in 1964, 1968, and 1975 by types

Type and crop year	Acreage	Yield per acre	Production
	1,000 acres	pounds	million lbs.
Flue-cured (Types 11-14)			
1964	628	2,211	1,388
1968	533	1,841	981
1975	717	1,973	1,415
Fire-cured (Types 21-23)			
1964	32	1,716	55
1968	23	1,689	39
1975	23	1,601	37
Burley (Type 31)			
1964	307	2,022	620
1968	238	2,372	563
1975	282	2,265	639
Maryland (Type 32)			
1964	39	1,085	42
1968	29	1,100	32
1975	24	1,050	25
Dark air-cured (Type 35-37)			
1964	14	1,735	24
1968	11	1,757	19
1975	9	1,690	15
Cigar filler (Type 41-44)			
1964	31	1,683	52
1968	23	1,766	41
1975	14	1,663	23
Cigar binder (Type 51-55)			
1964	14	1,862	26
1968	9	1,821	17
1975	13	1,851	23
Cigar wrapper (Type 61-62)			
1964	14	1,530	21
1968	13	1,343	19
1975	5	1,409	8
Puerto Rican Filler (Type 46)			
1964	31	1,231	38
1968	6	1,271	8
1975	3	1,500	4
Total U. S. tobacco (Types 11-72*)			
1964	1,109	2,044	2,266
1968	885	1,941	1,718
1975	1,090	2,008	2,189

*Includes Perique
SOURCE: U.S. Department of Agriculture (*55*).

increase in the percentage consumption for males and females under 21 years old. Cigarettes are by far the largest single tobacco product.

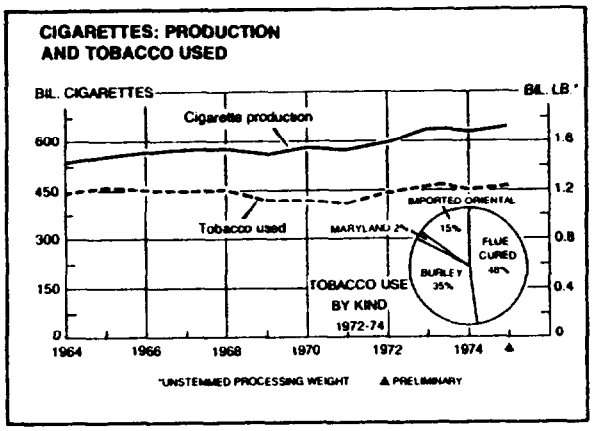

FIGURE 1.—In the United States flue-cured tobacco is the most important domestic type, with burley in second place. Note that cigarette production has increased while the tobacco used has remained about the same since 1964. This is due to use of stems, reconstituted sheets and filters in cigarette manufacture in recent years — formerly discarded as "waste".
SOURCE: Tso, T.C. (*27*).

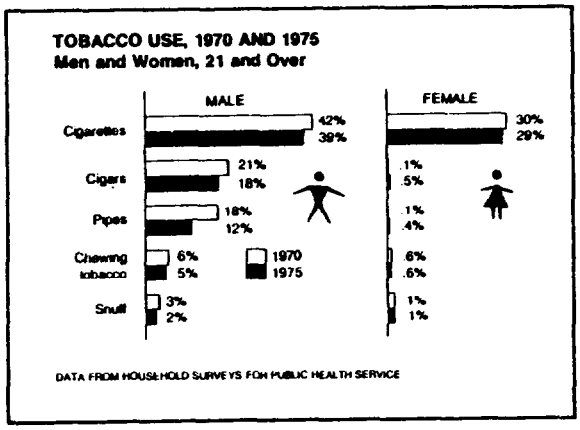

FIGURE 2.—Use of tobacco by men for cigarettes, cigars, pipes, chewing tobacco and snuff all showed a decrease in the 5-year period 1970–75. Use of tobacco by women also showed a slight drop in cigarettes, but a slight increase in use of cigars and pipes.
SOURCE: Tso, T.C. (*27*).

Types and Classes of Tobacco

There are at least 65 species within the genus *Nicotiana*. The species

Nicotiana tabacum L. is the main commercially grown species. This species has been established as a natural hybrid between *N. Sylvestris* and *N. Otophora* (*37*).

The types of tobacco generally used in smoking products are bright (flue-cured), Burley, Maryland, and cigar tobaccos, as well as oriental (aromatic) tobaccos. These types make up the bulk of the tobacco products (Table 1). Other types of tobacco exist, such as Perique, Latakia, and several Indian types, but they are not generally used in U.S. tobacco blends. Over the years, new varieties of bright, Burley, and other tobaccos have been developed that are multiple-disease resistant to specific tobacco diseases (*23*, *28*).

Within the species of *N. tabacum*, many varieties and types show wide differences in their chemical composition (*28*). Numerous germ plasms are available in the USDA collection, including approximately 1,000 tobacco introductions, 400 established varieties, and 100 breeding lines. Tso (*30*) reported that, in a preliminary examination of randomly selected samples from tobacco introductions, there was a threefold variation in sterol content, a tenfold variation in nitrate content, a thirtyfold variation in alkaloid content, and a fivefold variation in phenolic content. He concluded that greater variations probably exist among types not yet studied.

Based on methods of curing and the cultivar (a variety of tobacco within a tobacco type) used, leaf tobaccos produced in the United States are separated into the major classes shown in Table 2. There are five classes of air-cured tobacco including light air-cured, dark air-cured, and three kinds of cigar tobaccos: filler, binder, and wrapper (*26*, *28*). Filler is tobacco that makes up the bulk of a cigar, and wrapper is used for the outside covering. Binder is now used primarily for scrap chewing. Binding material for cigars is now made from reconstituted tobacco sheet (RTS). (RTS is also used in the manufacture of cigarettes, as will be discussed later.) Each of these tobaccos has specific characteristics and is produced for a specific purpose.

Under class, the subdivision is "types" (*26*, *27*), based on location of production, method of culture, and in most cases, plant cultivar. The cured leaf from each type is further subdivided into grade groups named on the basis of either principal use in manufacture or stalk position under the U.S. Government grading system. Each of the subdivisions is composed of several grades, determined by several elements of quality, such as body, texture, and color.

Physical and Chemical Characteristics

In addition to the genetic makeup, environmental factors, including mineral nutrition, soil properties, moisture supply, temperature, and light intensity, affect the chemical composition and physical properties of the leaf (*26*, *28*). The relationships among these factors and the

TABLE 2.—Classes and types of tobacco established by the U.S. Department of Agriculture

Type of curing and class	Type no.	Type name or locality
Flue-cured, Class 1	11A	Old Belt-Virginia and North Carolina
	11B	Middle Belt-Virginia and North Carolina
	12	Eastern North Carolina
	13	Border Belt-Southeastern North Carolina and South Carolina
	14	Georgia and Florida
	21	Virginia
Fire-cured, Class 2	22	Eastern-Kentucky and Tennessee Western-Kentucky and Tennessee
Air-cured		
Class 3A (light air-cured)	31	Burley
	32	Maryland
Class 3B (dark air-cured)	35	One-Sucker
	36	Green River
	37	Virginia Sun-Cured
Class 4 (cigar filler)	41	Pennsylvania Seedleaf, or Broadleaf
	42	Gebhardt
	43	Zimmer Spanish
	44	Little Dutch
	46	Puerto Rico
Class 5 (cigar binder)	51	Connecticut Broadleaf
	52	Connecticut Havana Seed
	53	New York and Pennsylvania Havana Seed
	54	Southern Wisconsin
	55	Northern Wisconsin
Class 6 (cigar wrapper)	61	Connecticut Valley Shade-Grown
	62	Georgia and Florida Shade-Grown
Miscellaneous, Class 7	72	Louisiana Perique
	77	Domestic Aromatic

SOURCE: U.S. Department of Agriculture (*36*).

tricarboxylic acid (TCA) cycle help define the smoking quality of tobacco leaves (*3*).

Smoking quality of tobacco leaf is determined to a great extent by the balance between the carbon and the nitrogen fractions (*28*). Atmospheric CO_2 is assimilated by the tobacco leaf through photosynthesis, while nitrogen is accumulated by the roots from the soil. The net result of nitrogen assimilation is, therefore, the utilization of a portion of newly photosynthesized carbon chains into the nitrogenous pool. Thus, when the nitrogen supply is abundant, more amino acids and nicotine and less sugar and starch will be synthesized. If the nitrogen supply is limited, acetate will accumulate from the TCA cycle and increase the production of carbohydrates, fats, volatile oils, resins, and polyterpines (*26, 28*). These variations will effect the resulting leaf

TABLE 3.—Approximate composition of freshly harvested tobacco leaves

Constituents	Bright cigarette tobacco	Cigar filter tobacco
	%	%
Carbohydrates	23.0	3.0
Protein	12.2	17.3
Soluble N compounds	3.3	6.7
Inorganics	12.0	14.0
Cellulose and lignin	10.0	9.5
Pentosans	2.0	3.0
Pectins	7.0	7.0
Ether-soluble resins	7.5	7.0
Tannins	2.0	2.5
Organic acids	13.0	13.0
Not identified	8.0	17.0

SOURCE: Frankenburg, W.C. (*7*).

texture, color, porosity, and combustibility. Examples include those tobaccos used in cigarette production, Turkish and bright (flue-cured), as well as cigar tobacco types. The Turkish tobacco is produced with limited supplies of nutrients and water, thus giving leaves more hydrocarbons and highly aromatic qualities (*26*). Cigar tobacco is grown with an abundant nitrogen supply yielding leaves high in protein and nicotine levels. Flue-cured tobacco is intermediary but slightly toward the carbon side. Table 3 illustrates typical differences among major constituents of bright and cigar tobacco leaves at harvest, and Table 4 describes the ranges of various constituents of the four main tobaccos used in cigarette production. Other environmental factors, such as the time of topping and the amount of sunshine (*27*), also play a role in the carbon-nitrogen balance.

The lower right portion of Figure 1 indicates that bright (or flue-cured) tobacco is the most widely used domestic type in the United States, while Burley, a light, air-cured type, ranks second in importance. Together, they account for most of the tobacco used. Typical values are flue-cured (45–75 percent), Burley (15–45 percent), Turkish (5–13 percent), and Maryland (1–7 percent) tobaccos (*26*). Some RTS is also used (*15–17*). The Standard Experimental Blend (SEB) used in the National Cancer Institute's experimental cigarettes, based on 1970 sales-weighted averages, are comparable (*15–17*).

The physical and chemical characteristics of tobacco leaf and smoke are unavoidably related to one another. Recent studies, particularly with bright tobaccos, show that characteristics such as leaf thickness, rate of leaf burn, and moisture content are significantly correlated with combustibility. Factors that promote good burning will generally

TABLE 4.—Range of chemical composition of tobacco being used in cigarettes*

Constituents	Flue-cured	Burley	Maryland	Oriental
Total nitrogen	1.00–3.00	1.50–4.50	1.25–3.00	1.40–3.50
Protein nitrogen	0.40–1.30	0.50–2.40	0.70–1.50	0.75–1.30
α-Amino nitrogen	0.08–0.45	0.10–0.50	0.08–0.36	0.10–0.54
Nicotine	0.80–3.50	0.40–4.50	0.65–2.00	0.50–1.30
Petroleum ether extractive	3.00–7.50	2.50–6.00	3.50–6.50	3.50–7.00
Starch	1.75–8.00	0.50–3.00	1.00–3.50	1.90–10.00
Soluble sugars	6.00–32.00	0.10–1.50	0.50–1.50	3.00–19.00
Nonvolatile acids**	9.00–26.00	15.00–38.00	13.00–25.00	16.00–23.00
Water-soluble acids**	2.50–5.00	0.30–3.50	0.40–3.50	-
pH (not %)	4.40–5.70	5.20–7.50	5.30–7.00	4.90–5.25

*Ranges in %.
**Milliliters of 0.1 *N* alkali per gram tobacco.
SOURCE: Darkis, F.R. (*2*).

result in lower levels of TPM in smoke, lower nicotine, cresols, volative phenols, hydrogen cyanide, and benz(*a*)anthracene, but will yield higher levels of acetaldehyde, acrolein, and carbon monoxide. The position of tobacco leaves on the stalk is known to influence greatly the resultant smoke characteristics (*37*). Present evidence shows that for higher leaf positions on the stalk, the combustibility is lower, the filling value of the tobacco is less, and the TPM, nicotine, HCN, volatile phenols, and polynuclear aromatic hydrocarbons in the mainstream smoke are higher. Thus, stalk position is an important indicator of both physical and chemical properties of the leaf and aids in interpreting precursors of the final product between leaf and smoke components. Table 5 shows some typical relationships between leaf characteristics and position on the stalk (*8*, *26*, *37*). Table 6 relates the effect of stalk positions and smoking properties (*27*). Similar data have been described by Wolf (*37*).

Culture and Harvesting Practices

Wolf (*37*) has reviewed the practices employed in tobacco culture and harvesting. A standard field practice with all domestic types of tobacco plants (except shadegrown cigar wrappers) is topping (removal of early blossoms) and suckering (removal of secondary buds) to promote the proper development in leaf size and thickness.

Priming (the removal of mature leaves at successive intervals) results in the maximum yield and quality from tobacco plants since leaves at different stalk positions mature at different stages. Depending on the type of tobacco plant and the weather conditions during harvest, there may be as many as nine primings.

Stalk-cutting is another method of harvesting, involving cutting the plant at the lowest stalk position and harvesting the entire plant at one

TABLE 5.—Stalk positions and leaf characteristics

Properties of Tobacco Types	Lower Leaves	Middle Leaves	Upper Leaves*
Flue-cured tobacco			
Cell membrane substances	Comparatively Higher	Comparatively Lower	Comparatively Lower
Total sugar	Lower	Higher	Lower
Total acid	Higher	Lower	Medium
α-amino N	Higher	Lower	Higher
Nicotine	Lower	Medium	Higher
Water-soluble N, total N	Medium	Lower	Higher
Soluble ash	Higher	Lower	Medium
Tannins, resins	Lower	Higher	Higher
pH	Higher	Lower	Lower
Air-cured Burley			
Color	Lighter	Darker	Darker
Porosity	More	Less	Less
Density	Lighter	Heavier	Heavier
Ammonium N, amino N, amido N	Lower	Medium	Higher
Nicotine N	Lower	Medium	Higher

*Not including uppermost tips.
SOURCE: Harlan, W.R. (*8*), Tso, T.C. (*27*).

TABLE 6.—Stalk positions and smoking properties

Smoking properties	Lower leaves	Upper and middle leaves
Strength (N compounds)	relatively light	relatively strong
Aromaticity (tannins, resins)	aromatic	highly aromatic
Mildness (sugars, starch, oxalic acid) and sharpness (cell membrane substances, ash constituents, citric acid)	somewhat sharp	mild

SOURCE: Harlan, W.R. (*8*), Tso, T.C. (*27*).

time. In general, Burley and Maryland tobaccos are harvested by stalk-cutting.

The application of herbicides to control weeds, fertilizers to enhance plant growth, pesticides to treat soil and control plant diseases, and insecticides may directly or indirectly leave residues on plant material; this factor must be considered when the characteristics of the tobacco leaf and smoke chemistry are examined.

Curing and Aging

The green tobacco leaf primed from the plant goes through a process known as "curing" in order to develop desirable taste and aroma for

smoke products. Several different curing processes are used to produce leaf tobacco suitable for the manufacture of a variety of tobacco products (*37*).

Curing is a process during which chemical conversions take place in the tobacco leaf. During flue-curing or air-curing, chemical conversion is dominated by hydrolytic enzymes. Disaccharides and polysaccharides are hydrolyzed to simple sugars; proteins are hydrolyzed to amino acids which undergo subsequent oxidative deamination; pectins and pentosans are at least partially hydrolyzed to pectic acid, uronic acid, and methanol. A second step occurs only in air-cured tobaccos and includes conversions such as the oxidation of simple sugars to acids, the oxidation and polymerization of certain phenolic compounds, and some decrease in alkaloids and dry weight (*26*).

As a result of years of research, numerous advances have been made in the procedures used to harvest, cure, and process tobacco. One particular development in the early 1950's was the process of manufacturing reconstituted tobacco sheets (out of tobacco scrap) in a manner analogous to paper manufacture (*13*). The process will be discussed later. The significance of the process lies in the fact that tobacco need not be harvested and cured in whole leaf form, thus suggesting new mechanized approaches to harvesting and curing.

A new curing procedure called homogenized leaf curing (HLC), developed by scientists at the U.S. Department of Agriculture, involves the homogenization, incubation, and dehydration of tobacco leaf (*4, 33*). The fundamental concept is to cause the necessary chemical changes to occur in a homogenized tobacco slurry instead of in the harvested whole leaf. The process saves considerable hand labor normally required for handling whole leaf, allows a mechanism for removal of undesirable components, and permits better control and enhancement of biochemical and chemical changes. Results have shown that the HLC method may provide smoking quality that is comparable to conventionally cured leaf but with a relatively lower biological response (*33*).

Cured, unaged tobacco is still unsuitable for manufacturing into tobacco products because it has a sharp, disagreeable odor and an undesirable aroma and produces irritating smoke with unacceptably harsh flavor (*26*). To improve these conditions, cigarette tobaccos (flue-cured, Burley, Maryland and Turkish) are subjected to a further process called aging. Aging greatly improves the aroma and other qualities desirable in smoking products. The aging process can be natural or forced, depending upon time, temperature, and humidity. A 1- to 2-year aging period is not unusual for cigarette tobaccos.

The treatment of cigar tobaccos consists of two steps (*7*). The first step is storage and the second is fermentation. Current knowledge of the chemical conversions during aging and fermentation is rather limited (*26*). The most noticeable chemical changes in the aging process

are an increase in volatile acids and a decrease in α-amino nitrogen. Flue-cured and Turkish tobaccos also exhibit a loss of reducing sugars and volatile bases other than nicotine. In fermentation, new chemical reactions appear and ongoing reactions are intensified. A decrease in tobacco alkaloids, especially nicotine, is evident (*7*). Large amounts of ammonia are produced, and amide and α-amino nitrogen levels are decreased. The pH increases because of the elimination of organic acids through oxidation and decarboxylation. It is likely that enzymes, microorganisms, and catalysts all play a part in the fermentation process (*26*).

Representative analyses of aged and cured cigarette and cigar tobaccos are shown in Tables 7 and 8. These chemical variations are the results of different varieties, cultures, fertilizers, soils, climates, and post-harvesting practices as described above.

Other Factors

Leaves from different levels on the stalk possess considerably different chemical and physical properties. For example, upper leaves possess higher nicotine, lower total sugar, higher tannins and resins, lower ash, and higher total nitrogen; lower leaves tend to contain higher total acid, higher soluble ash, and higher pH. However, not all substances are at their highest or lowest concentration in the upper and lower leaves. The leaves at the middle stalk position, for example, have the highest sugar, lowest α-amino nitrogen, lowest total acid, lowest total nitrogen, and lowest soluble ash. Selecting mature leaves at various time intervals (priming) allows maximum use of tobacco leaves and selectivity in future blending.

Because of the chemical and physical differences, leaves from various stalk positions also vary in smoke characteristics, as shown in Tables 5 and 6. Lower leaves usually deliver a lighter "strength," somewhat sharper taste, and less aromatic smoke than the upper and middle leaves (*1*). These smoking properties are largely functions of chemical composition. For example, nitrogen compounds are believed to be associated with strength; tannins and resins are associated with aromaticity; sugars, starch, and oxalic acid are associated with mildness; and cell membrane substances, ash constituents, and citric acid are associated with "sharpness" (*1*). Certain physical quality factors are also related to chemical components, as all these variables are interrelated. In a recent study with bright tobaccos (*31*), many physical variables including leaf thickness, rate of burning, leaf color, moisture content, moisture equilibrium, specific volume, and trichome numbers were found to be significantly correlated with many leaf chemical variables.

The presence of radioelements, including radium-226, lead-210 and polonium-210 have been reported in tobacco and tobacco smoke (*19*) and reviewed recently by Harley and coworkers (*9*). Contents of Po^{210} in

TABLE 7.—Representative analyses of cigarette tobaccos (leaf web after aging, moisture-free basis)

Component %[a]	Flue-cured. Type 13	Burley. Type 31	Maryland. Type 32	Turkish[b]
Total volatile bases as ammonia	0.282	0.621	0.366	0.289
Nicotine	1.93	2.91	1.27	1.05
Ammonia	0.019	0.159	0.130	0.105
Glutamine as ammonia	0.033	0.035	0.041	0.020
Asparagine as ammonia	0.025	0.111	0.016	0.058
α-Amino nitrogen as ammonia	0.065	0.203	0.075	0.118
Protein nitrogen as ammonia	0.91	1.77	1.61	1.19
Nitrate nitrogen as NO_3	trace	1.70	0.087	trace
Total nitrogen as ammonia	1.97	3.96	2.80	2.65
pH	5.45	5.80	6.60	4.90
Total volatile acids as acetic acid	0.153	0.103	0.090	0.194
Formic acid	0.059	0.027	0.022	0.079
Malic acid	2.83	6.75	2.43	3.87
Citric acid	0.78	8.22	2.98	1.03
Oxalic acid	0.81	3.04	2.79	3.16
Volatile oils	0.148	0.141	0.140	0.248
Alcohol-soluble resins	9.08	9.27	8.94	11.28
Reducing sugars as dextrose	22.09	0.21	0.21	12.39
Pectin as calcium pectate	6.19	9.91	12.41	6.77
Crude fiber	7.88	9.29	21.79	6.63
Ash	10.81	24.53	21.98	14.78
calcium as CaO	2.22	8.01	4.79	4.22
potassium as K_2O	2.47	5.22	4.40	2.33
magnesium as MgO	0.36	1.29	1.03	0.69
chlorine as Cl	0.84	0.71	0.26	0.69
phosphorus as P_2O_5	0.51	0.57	0.53	0.47
sulfur as SO_4	1.23	1.98	3.34	1.40
Alkalinity of water-soluble ash [c]	15.9	36.2	36.9	22.5

[a] In % except for pH and alkalinity.
[b] Blend of Macedonia, Smyrna, and Samsun types.
[c] Milliliters of 1N acid per 100 g tobacco.
SOURCE: Harlan, W.R. (*8*).

leaf tobacco and tobacco soil vary with the origin of the sample and methods of culture and curing (*24*). Polonium seems not to be entirely derived from radium. The plant probably takes it up from the soil or air. The general range of Po^{210} in tobacco leaf varies from 0.15 to 0.48 pCi/g (10^{-12} Curies per gram); in tobacco-growing soil, it varies from 0.26 to 0.55 pCi/g. The amount of Ra-226 in tobacco-producing soil appears to be related to phosphorus fertilization. Soils having high available P continuously used for tobacco crops usually have a higher Ra-226 content, the range being 0.52 to 1.53 pCi/g (*24*). The significance of these radioelements in tobacco and tobacco smoke is being extensively studied with Pb^{210}-enriched leaf tobacco by USDA.

TABLE 8.—Representative analyses of cigar tobaccos (leaf web after fermentation, moisture-free basis)

Component[a]	Conn. shade-grown wrapper. Type 61	Northern Wisconsin binder. Type 55	Penn filler. Type 41	Puerto Rican filler. Type 46	Cuban filler. Type 81	Sumatra wrapper. Type 82
Total volatile bases as ammonia	1.293	1.055	0.874	0.707	1.478	0.670
Nicotine	1.47	2.68	2.04	0.90	2.23	1.42
Ammonia	0.914	0.575	0.495	0.348	1.012	0.313
Total amide as ammonia	0.225	0.199	0.165	0.264	0.232	0.208
Protein nitrogen as ammonia	2.20	2.14	2.88	3.26	2.81	3.01
Total nitrogen as ammonia	5.78	4.75	5.16	4.65	5.83	5.17
pH	6.27	6.33	6.10	7.21	6.56	7.25
Ash	23.79	24.94	24.50	22.45	22.57	22.34
Alkalinity of water-soluble ash[b]	90.4	45.5	47.0	62.7	43.0	93.6

[a]In % except for pH and alkalinity.
[b]Milliliters of 1N acid per 100 g tobacco.
SOURCE: Harlan, W.R. (*8*).

Aflatoxin B_1, the most toxic of the four known aflatoxins, is produced by *Aspergillus flavus* Lk. ex Fr. The binding of aflatoxin B_1 to both native and denatured deoxyribose nucleic acid (DNA) partially explains its extreme toxicity and carcinogenicity. Aflatoxins have been reported to occur in many commodities, but its presence in leaf tobacco has not been positively confirmed, although *A. flavus* was known to be present in various grades of air-cured Burley tobacco. Certain types of tobacco contain higher populations of fungi than other types (*6*). These differences probably result from culture, curing, and handling practices as well as from the chemical composition of tobacco leaf and the climate in which it is grown. An examination of samples of leaf tobacco and of cigarette smoke condensate by Tso, et al. (*26*) failed to show aflatoxin B_1. Pure aflatoxin B_1 added to cigarettes was not recovered in the smoke condensate, indicating that aflatoxin B_1, even if present, was changed or decomposed during the smoking process.

Relationships Among Tobacco Leaf, Smoke, and Biological Response

Recent reports have been published dealing with precursor-product relationships among specific leaf tobacco components and smoke constituents (*20, 26, 31, 34*). One comprehensive study was conducted to examine the relationships among leaf, smoke, and biological responses using well-defined bright tobacco samples specially produced for this

purpose. This study involved a total of 151 variables, including 102 leaf and agronomic characteristics, 42 cigarette and smoke components, and 7 biological responses (*31*). The results clearly indicated that certain leaf characteristics could be used as "markers" to predict total smoke delivery or individual smoke components. These findings demonstrated that modification of these markers through genetic, cultural, or curing procedures might lead to the development of leaf tobacco of more desirable quality and usability.

The correlations made by Tso and coworkers may be interpreted in the sense of precursor-product relationships between specific leaf and smoke components and between certain smoke components and biological responses. Table 9 gives the correlations among some selected leaf and smoke variables.

Using the same selected leaf characteristics, the correlations with the results of seven short-term bioassay systems were determined as shown in Table 10. The sebaceous gland suppression system showed many significant and interesting correlations with certain leaf characteristics (*34*). In examining all these variables, the authors commented that one significant factor appeared to be the one which affects leaf combustibility and thus the formation of components that affect suppression. Variables that promoted combustion were generally negatively associated with suppression, and variables that inhibited combustion were generally positively associated with suppression. In addition, phenolic compounds were positively associated with suppression. These compounds may serve as precursors of smoke constituents with tumor-promoting activity.

In addition to the sebaceous gland suppression system, the *E. coli.*, virus-infected quail, and mixed cell-culture systems also used cigarette smoke condensate. These three systems did not demonstrate any meaningful correlations with the variables examined. Correlations among selected smoke and biological variables are shown in Table 11. For example, static burning rate was negatively associated, whereas total phenols, benzo(*a*)pyrene (B*a*P), benz(*a*)anthracene (BaA), and smoke pH were positively associated with sebaceous gland suppression. Tso, et al. (*34*) commented that it is somewhat surprising that dry total particulate matter, cresols, acetaldehyde, acrolein, and hydrogen cyanide did not show any statistically significant correlation with the biological data employing whole smoke in these studies.

Smoke delivery and smoke composition thus seem to depend on the characteristics of leaf tobacco (*26*). The effects of genetic and stalk position differences are reflected in botanical, physical, and chemical properties of leaf tobacco, which in turn are clearly illustrated in the smoke constituents of these experimental samples. These results agree with those of parallel studies using leaf "markers" for identification of leaf quality and usability as described by Tso and Gori (*32*). Usability in their definition represents the state of being usable without adverse

TABLE 9.—Correlations among smoke and leaf variables

	Static-burning rate (mg/min.)	Nicotine in smoke (mg/100 g tobacco smoked)	Dry TPM (g/100 g tobacco smoked)	Nicotine-free dry TPM (g/100 g tobacco smoked)	Acetal-dehyde (mg/100 g tobacco smoked)	Acrolein (mg/100 g tobacco smoked)	BaP (μg/100 g tobacco smoked)	BaA (μg/100 g tobacco smoked)	HCN (mg/100 g tobacco smoked)	Phenols (mg/100 g tobacco smoked)	o, m, p-cresols (mg/100 g tobacco smoked)	Total vol. phenols (μg/g tob. smoked)	Smoke pH (last puff)
Trichoine	-.604**	.450**	.705**	.719**	-.122	-.484**	.538**	.494**	.665**	.744**	.836**	.142	.399*
Leaf thickness	-.403*	.587**	.462**	.399*	-.577**	-.594**	.353*	.308	.543**	.686**	.530**	-.088	.695**
Fire-holding capacity	.684**	-.612**	-.799**	-.792**	.407*	.663**	-.663**	-.548**	-.755**	-.827**	-.820**	-.179	-.599**
Moisture equilibrium	-.671**	.468**	.672**	.675**	.089	-.158	.594**	.587**	.488**	.668**	.725**	.712**	.340
pH (leaf tobacco)	.680**	-.538**	-.601**	-.575**	.382*	.548**	-.597**	-.571**	-.634**	-.699**	-.671**	-.688**	-.599**
K	.615**	-.754**	-.804**	-.761**	.550**	.608**	-.662**	-.566**	-.766**	-.801**	-.703**	-.775**	-.699**
Cell-wall substance	.398*	-.212	-.406*	-.425*	-.095	.144	-.460**	-.490**	.278	-.433*	-.565**	-.511**	-.199
Total N	-.662**	.905**	.884**	.81!**	-.308	-.426*	.793**	.760**	.831**	.949**	.824**	.919**	.852**
Nitrate N	.367*	-.280	-.451**	-.461**	.167	.382*	-.224	-.114	-.431*	-.498**	-.543**	.099	-.261
Total alkaloid (dist.)	-.526**	.984**	.710**	.595**	-.368	-.297	.656**	.637**	.467**	.832**	.581**	.744**	.929**
Total vol. bases	-.513**	.985**	.758**	.650**	-.359*	-.333	.672**	.650**	.723**	.864**	.626**	.781**	.924**
α amino N	.603**	.475**	.472**	.439*	-.073	-.175	.423*	.450**	.569**	.496**	.427*	-.090	.483**
Total free amino acids	-.445**	.263	.555**	.588**	.263	-.535**	.449*	.427*	.605**	.552**	.622**	.591**	.312
Arginine	-.410*	.233	.476**	.503**	.233	-.690**	.344	.302	.587**	.447*	.511**	.489**	.275
Aspartic acid	.609**	-.358*	-.529**	-.534**	.324	.459**	-.471**	-.436**	.466**	.488*	-.551**	-.528**	-.294
Proline	-.560**	.364*	.382*	.360*	-.192	-.530**	.348	.319	.356*	.425*	.580**	.508**	.121
Dimethylamine	-.559**	.573**	.497*	.444*	-.113	-.196	.523**	.522**	.468**	.541**	.460**	.523**	.548**
Total polyphenols	-.474**	.151	.507**	.560**	.161	-.169	.538**	.514**	.399*	.496**	.642**	.563**	.163
Chlorogenic acid	-.585**	.561**	.634**	.610**	-.084	-.100	.690**	.645**	.468**	.653**	.550**	.624**	.527**
Rulin	-.444*	.147	.495**	.548**	.245	-.036	.434*	.364*	.348	.452**	.610**	.529**	.077
Scopoletin	-.728**	.620**	.748**	.727**	-.456**	-.735**	.620**	.574**	.738**	.801**	.785**	.821**	.645**
Lignin	-.140	.378*	.528**	.529**	.016	-.036	.392	.393*	.570**	.396	.328	.362*	.241
Oxalic acid	.545**	.516**	.596**	.575**	-.623**	-.723**	.584**	.479**	-.739**	.646**	.618**	.626**	.578**
Malic acid	.452**	-.481**	-.748**	-.763**	.112	.412*	-.583**	-.510**	-.657**	-.729**	-.732**	-.765**	-.437*
Pentadecenoic acid	-.449*	.410*	.659**	.675**	.035	-.140	.590**	.589**	.567**	.500**	.548**	.529**	.229
Stigmasterol	.520**	-.565**	-.543**	-.501**	.761**	.820**	-.484**	-.429**	-.627**	-.596**	-.508**	-.558**	-.659**
p,p'-TDEE	-.366*	.667**	.636**	.584**	-.205	-.321	.454**	.447*	.639**	.699**	.584**	.665**	.550*
Total DDT + TDE	.228	.378*	.533**	.534**	.034	-.070	.472**	.460**	.519**	.485**	.517**	.519**	.278
Aroma	-.364	.531**	.583**	.332	.211	.096	.566**	.527**	.328	.525**	.501**	.528**	.358*
Flavor	-.221	.470**	.566**	.430*	.313	.212	.533**	.500**	.280	.509**	.512**	.533**	.284
Strength	.416*	.627**	.714**	.514**	.054	.023	.585**	.514**	.546**	.696**	.623**	.700**	.488**

* - 5 0/0 significance
** - 1 8/0 significance
SOURCE: Tso, T.C. (34).

TABLE 10.—Correlations among selected leaf and biological variables

Variable	Sebaceous gland	*E. Coli* zone inhibition	Virus-infected quail	Mixed cell culture	Cilia toxicity	Cyto-toxicity	Macro-phage
Stalk position	0.506**	-0.090	-0.009	-0.316	-0.037	-0.076	-0.023
Trichome	.391*	-.169	.007	-.327	-.158	-.111	-.038
Leaf thickness	.352*	.060	.156	-.313	.295	-.373*	-.004
Rate of burn	-.554**	.011	-.083	.193	-.034	.017	.091
Moisture equilibrium	.466**	-.100	.056	-.460**	.048	.080	-.054
pH (leaf tobacco)	-.494**	.104	-.284	.209	-.039	.154	-.152
Potassium	-.523**	-.106	-.221	.070	-.066	-.016	.043
Total nitrogen	.595**	-.086	.200	-.194	.037	-.096	.171
Nitrate nitrogen	-.473**	.015	.148	.205	.035	.083	.092
Total alkaloids	.439*	-.053	.219	-.124	.255	-.150	.166
Total volatile bases	.458**	-.081	.229	-.089	.140	-.130	.175
α-Amino nitrogen	.178	-.303	.204	.064	-.306	-.100	.247
Total free amino acids	.355*	-.239	-.012	-.087	-.304	-.111	.053
Aspartic acid	-.337	-.048	-.107	.172	-.168	.002	.134
Dimethylamine	.451**	.394*	-.042	.330	.017	-.133	.185
Total polyphenols	.382*	-.223	.148	-.353*	-.197	.001	-.046
Chlorogenic acid	.509**	-.025	.160	-.326	.086	-.050	.098
Scopoletin	.488**	-.076	.044	-.264	.077	-.181	.085
Oxalic acid	.397*	-.039	.401*	.028	-.130	-.014	.104
Malic acid	-.507**	-.117	-.072	.224	.223	.020	.105
Pentadecenoic acid	.196	-.123	.143	.064	-.375*	.274	-.106
Stigmasterol	-.361*	-.070	-.171	-.101	-.171	.225	-.043
Total DDT + TDE	.460**	.030	.180	-.186	-.271	.102	.159
Flavor	.358*	-.126	-.010	-.249	-.065	.020	-.178
Strength	.428*	.147	.048	-.272	-.126	.144	.126

* and ** — significantly different from 0 at 5 and 1 percent, respectively.
SOURCE: Tso, T.C. (*26*).

$$\text{Usability index} = \frac{A}{B}$$

If chemical, physical and botanical characteristics are considered:

$$\text{Usability index} = \frac{A}{B} + \frac{C+D}{E}$$

where

A = nitrate + K + total ash + cellulose,
B = nicotine + TVB + α-amino nitrogen + starch + polyphenols + PEE + lipid residues + waxes + phytosterols + fatty acids,
C = filling value + combustibility,
D = stem/lamina ratio,
E = thickness.

(TVB and K = total volatile bases, potassium) PEE = petroleum ether extracts

TABLE 11.—Correlations among selected smoke and biological variables

Variable[1]		Sebaceous gland	*E. Coli* zone inhibition	Virus-infected quail	Mixed cell culture	Cilia-toxicity	Cyto-toxicity	Macro-phage
Static burning rate per minute	mg	-0.465**	0.010	-0.145	0.390*	-0.128	0.030	-0.132
Dry total particulate matter[2]	g	.272	.234	.073	.104	.272	-.017	-.104
Nicotine in smoke[2]	mg	.268	.171	.204	-.013	.472**	-.152	-.196
o-, *m*-, and *p*-Cresols[3]	mg	.137	.116	-.074	.035	.293	-.167	-.314
Total volatile phenols[2]	mg	.542**	-.165	.054	-.322	.011	-.142	.080
Acetaldehyde[3]	mg	-.104	-.112	-.329	-.033	-.216	.180	-.018
Acrolein[3]	mg	.073	-.109	-.089	.109	-.308	.263	.145
Hydrogen cyanide[3]	mg	.138	.152	.280	.163	.125	-.078	-.130
Benzo[*a*]pyrene[2]	μg	.388*	.249	.205	.019	.251	-.014	.057
Benzo[*a*]anthracene[2]	μg	.446*	-.098	.291	-.024	-.170	-.064	.025
Smoke pH (last puff)	pH	.468**	-.034	.213	-.103	.345	-.362*	.228
Carbon monoxide[2]	mg	.285	.105	.373*	.002	-.444*	.264	-.128
Carbon dioxide[2]	mg	.323	.136	.312	.031	-.335	.194	-.178

[1]* and ** = significantly different from 0 at 5 and 1 percent, respectively.
[2]per gram tobacco burned
[3]per 100 grams tobacco burned
SOURCE: Tso, T.C. (*26*).

effects. Markers were used to establish a "usability index." High emphasis was placed on the chemical constituents. Physical factors were next in importance because they can be improved through reconstitution. Botanical factors were considered only when natural leaf was used and entire stems were returned for cigarette manufacture.

Thus, the potential is there to assume that modification of the markers identified in this type of analysis may lead to the improvement of the smoke products as well as the biological effects of the smoke.

Modification of Tobacco and Tobacco Products

It has been reported by Tso and coworkers (*33*) that the labor of tobacco harvest and post-harvest handling may account for 50 to 55 percent of the total required to produce the crop. Consequently, many attempts have been made to reduce use of hand labor. It is not essential that the tobacco leaf be kept whole in order to be useful to the tobacco industry (*14*). Tso and coworkers (*4*, *33*) recently reported the results of a new procedure for curing leaf tobacco through homogenization, incubation, and dehydration, called homogenized leaf curing (HLC). The objectives of the HLC process were threefold: to reduce production labor costs, to reduce or eliminate undesirable factors that may be associated with the smoking and health problem,

and to improve tobacco usability by enhancing certain physical and chemical factors. Preliminary results (*4*, *33*) suggest HLC advantages are the capability for more complete mechanization and the enhanced potential for reduction or elimination of substances found to be hazardous to health. Reductions in total volatile bases, nicotine, reducing substances, total particulate matter, and nitrosamines have been reported (*33*).

Another method of modifying tobacco and tobacco products involves development of the reconstituted tobacco sheet (RTS); this method has been reviewed by Moshey (*14*) and Mattina and Selke (*13*). The original impetus for developing a reconstitution process was purely economical. For each pound of auction weight tobacco, only about 63 percent was usable shredded leaf tobacco, although approximately 6 percent of the stem material was also blended in smoking tobacco. The remaining 31 percent, consisting of sand (2 percent), discarded stems (18 percent), manufacturing fines (1 percent), and moisture and aging loss (10 percent) was lost to the manufacturer. A process that could utilize the lost stems and fines and control moisture would increase the amount of usable tobacco from a harvest, cut costs, and offer some manufacturing control over the physical and chemical properties of the resultant product (*13*).

Several processes were developed in the early 1950's. These were of two general type groups; in one group, the tobacco is ground into fine particles, mixed with a hydrocolloid gum, and cast on an endless steel belt. The other, more widely used group of processes, involves mechanically working the insoluble portion of the tobacco into a fibrous mass and forming it, via papermaking techniques, into a web. In one variation of the paper process, the soluble portion is diverted prior to the papermaking and then added back to the self-supported web. In another variation, the soluble portion remains with the fibrous material throughout the processing. For all processes, the finished product is in the form of leaflets which are then blended with natural tobacco and shredded.

The significance of the sheet process lies in the ability to chemically and mechanically produce desired changes during the pulping process. For example, chemical extractions can be performed to reduce nicotine and other constituents. Tar-yield levels can be reduced to some extent, and additives can be put into the material. The structural modifications which can be effected through reconstituted sheet technology could result in considerable differences in the burn properties and in the smoke. Produced tobacco sheet with a 10 mg/cigarette tar yield without filtration is now available using RTS technology. Lower figures are possible but may cause the sheet to be undesirable as a tobacco product. Flavorings and other additives can also be added at selective stages during the process if necessary, depending upon the solubility and volatility of the additive.

The components of leaf tobacco can be classified into three different categories. Some components are essential for smoke quality and desirability, others have either little or no effect, and a third category consists of components that serve as precursors of undesirable smoke constituents such as HCN and aza-arenes (*5, 28*).

One class of components in the third category is fraction-1-protein (*12, 28, 29*). This and other proteins do not contribute in any significant way to smoke aroma or flavor. Removal of fraction-1-protein achieves two purposes—improved leaf quality and usability, and fraction-1-protein as a potential food source. It is estimated that up to 6 percent of the tobacco yield could be used for feed and food purposes (*28*).

Fraction-1-protein is the major soluble protein of green plants and may account for 50 percent of the soluble protein fraction and 25 percent of the total protein (*26, 28*). The protein is an enzyme called carboxydismutase (*21*) that catalyzes the first step in the transformation of CO_2 into carbohydrates during photosynthesis (*28*).

Tso (*33*) and DeJong (*4*) have reported that the fraction-1-protein can be removed for beneficial use by the above-mentioned HLC process, and could be used as a food source for millions of people annually (*28*). The protein has been evaluated as a food source (*28, 29*) and found to compare favorably with egg and human milk for essential amino acid content.

Cigarette Engineering

The tobacco blend can vary in the amount of Burley, bright (Virginia), Maryland, and oriental leaf and in the amount of reconstituted tobacco sheet used. Casing solutions are used to hold the tobacco blend together. Humectants (moisture retainers) are added to maintain the necessary body and moisture qualities and to contribute to the flavoring of the blend. Flavor-enhancing additives are used to make the smoke pleasant and more acceptable to the smoker. To maintain the physical integrity of the product, a paper wrapper is used. Each of these ingredients may affect the burn rate, puff number, pyrolysis products, and ultimately the chemical constituents of mainstream and sidestream smoke and smoke condensate.

Typical casing materials that may be used are sugars, sirups, licorice and balsams. These additives improve or change the flavor characteristics and burning qualities and impart important binding qualities to the blend. However, additives, when pyrolyzed, may yield undesirable as well as desirable products. Licorice, for instance, could be a precursor of polyaromatic hydrocarbons (PAH). Sugars used in casings cause an increase in furfural, nicotine, and tar in resulting smoke and a decrease in volatile acids (*21*).

Flavoring agents are added at different steps in the cigarette manufacturing process, depending upon volatility. Volatile flavors, such as alcohol-soluble fruit extractives, menthol oils, and aromat