There is a marked variation in the number of cigarettes smoked per day by different smokers. Some individuals smoke less than one cigarette per day and others smoke 60 or more cigarettes per day (Pierce and Hatziandreu 1989; Burns and Pierce 1992). The day-to-day variation in number of cigarettes smoked per day by an individual smoker is much smaller than the variation among different smokers in the population, particularly for those who smoke every day. The number of cigarettes smoked per day varies somewhat with age, gender, and socioeconomic factors. There is also some variation according to racial grouping. Black smokers tend to report fewer cigarettes smoked per day than white smokers, and Hispanic smokers are more likely to be occasional smokers.

An extensive presentation of the variation in smoking topography is presented in the Surgeon General's report on nicotine dependence (DHHS 1988), and the variation in the topography of smoking as measured in published studies is presented in Table 1. There is relative uniformity in the mean values for the measures of smoking topography across these studies; but there is a substantial variation in the measures of smoking topography among individual smokers (Nil 1986; Guyatt 1989; Bridges 1990; Russell *et al.*; Battig 1982). This variation among individual smokers is defined by the standard deviation of the measured values presented in Table 1, and it is evident that there is a wide variation in the pattern of inhalation among individual smokers. The variation in pattern of smoking is much less for two cigarettes smoked by the same smoker (Battig 1982), suggesting that it was differences between smokers in the way that they smoked, rather than differences in the way a specific smoker smoked sequential cigarettes, that produced the variation in smoking topography found by these studies.

The pattern of smoking also varies with the tar and nicotine yield of the cigarette smoked. Some smokers of lower yield cigarettes clearly compensate for the lower yield of these cigarettes by altering the way they smoke these cigarettes to increase the absorption of nicotine. Their nicotine absorption levels are substantially higher than would be predicetd from the nicotine yields generated from machine smoking (Benowitz 1983; Kolonen 1991; Hofer 1992). This compensation is largely accomplished by changes in puff volume and frequency, rather than by increasing the number of cigarettes smoked per day (Kolonen 1991; Battig 1982; Bridges 1990).

A number of investigators have examined changes in the topography when smokers switch to lower yield cigarettes, both immediately and after a period of regular smoking of these cigarettes. When smokers switch to a cigarette that has a lower yield than their regular brand, there is an immediate compensation in the smoking topography with an increase in puff volume and puff frequency (Kolonen 1991; Woodman 1987; Guyatt

1989). However, the change in puff frequency can revert to the original rate within a few weeks or months (Guyatt 1989). In the studies that measured nicotine absorption, there was a fall in amount of nicotine absorbed when the smoker switched to a lower yield cigarette, but the magnitude of the fall was much smaller than would be predicted from the machine smoking results.

Existing data clearly indicate that differences in cigarette manufacturing processes which affect the yield of the cigarette can influence smoking topography. Many of the changes appear to be a compensatory repsonses intended maintain nicotine absorption from cigarettes with a lower nicotine yield, such as increased puff volume and inhalation depth. Topography alterations may also result from changes in draw resistance (Guyatt 1988), filter perforations (Kozlowski 1988), and possibly other differences in manufacturing technology.

MARKERS OF THE DOSE ABSORBED BY THE SMOKER

Chemical analyses can quantify the several thousand constituents present in tobacco smoke (Chapters B and D), but it is neither practical nor technically possible to measure human absorption of each of the several thousand smoke constituents. Therefore, evaluation of the relative toxicities of the smoke produced by low ignition-potential cigarettes will need to rely heavily on selected chemical analyses and biological (*in vitro* and *in vivo*) toxicity tests of the whole smoke and its components. Measures of absorption can be used predominantly as markers of whole smoke exposure.

Biochemical markers, such as nicotine and carbon monoxide, can quantitatively estimate the amount of smoke absorbed by the smoker and effectively integrate the sometimes competing effects of differences in cigarette manufacturing and resultant changes in smoking topography. Biochemical markers produce a unifying estimate of the differences in exposure/dose resulting from smoking different brands of cigarettes. If switching to low ignition brands leads to a greater number of cigarettes being smoked per day, a deeper inhalation, or a change in the topography of smoking, it may lead to greater smoke absorption; these would increase the risk of those adverse health effects summarized in Chapter A. A number of biochemical markers have been used to quantify the amount of smoke inhaled and retained by the smoker (IARC 1986, DHHS 1988).

The accuracy and utility of using a single biochemical marker to estimate the absorption of whole smoke is influenced by several factors. First is the quantitative relationship between an increase in the level of the marker and the amount of the smoke absorbed by the smoker. Second is the specificity of the marker for the absorption of tobacco smoke as compared to other

sources of the marker. Third is the direct quantitative relationship between the absorption of the marker and the absorption of other toxic constituents in the smoke. And finally, there is the precision of the measurements.

Biochemical markers of absorption can be used under non-experimental conditions that represent more typical smoking behavior. Biochemical markers are advantageous since they can be sampled after a period of smoking under non-experimental conditions approximating normal behavior. This may be less disruptive to the smoking behavior than topographical measures taken during smoking by attaching instrumentation to the cigarette or to the human smoker.

Carbon Monoxide

Carbon monoxide (CO) is a toxic gas phase constituent of tobacco smoke and is a useful measure of the depth of inhalation of cigarette smoke. The CO produced is dependent on the amount of oxygen available to the burning tobacco. However, other factors, such as density of the tobacco, paper porosity, puff volume, and draw rate, may influence the amount of CO produced and reduce the correlation between CO and particulate matter (IARC 1986). Therefore, CO can not be predicted on the basis of particulate matter (or tar) yield.

CO binds reversibly to hemoglobin (the oxygen-carrying protein in the blood), and is cleared from the body primarily by exhalation. The concentration of carbon monoxide in the blood can be determined as the partial pressure of CO in the expired air or as the level of carboxyhemoglobin in the blood (DHHS 1988; IARC 1986). The blood and breath levels of CO vary markedly during a smoker's day due to the intensity of smoking in the hours immediately before the measurement and the short 4-hour half-life of CO in the blood.

The single point-in-time measurement of blood or breath CO is very precise, but may not represent the total daily smoke absorption due to its short half-life, the background sources of CO and endogenous production of CO (Woodward 1991). However, the change in blood or breath CO level after smoking a single cigarette can represent the smoke absorbed and may be a better measure of the depth of inhalation of the cigarette smoke than serum nicotine or cotinine levels. Since CO is absorbed almost exclusively by the lung, it is a more specific measure of smoke inhalation than nicotine, which is also absorbed through the oral mucosa (Herling 1988). CO is also a marker for exposure to the gas phase constituents of smoke since it is present in only the gas phase.

Nicotine and Cotinine Levels

Concentrations of nicotine and its longer half-life metabolite, cotinine, can be measured in the blood, saliva and urine to very low levels (DHHS 1988; IARC 1986; Anderson 1991; Benkirane 1992; Etzel, 1990; Feyerabend 1990; Weinhold 1987). The major non-tobacco sources of nicotine are nicotine patches and gums used as adjuncts to smoking cessation. Nicotine is not produced by the body and is found in only trace amounts in plants or foods other than tobacco (DHHS 1990). The contribution of environmental tobacco smoke to the cotinine level of most regular smokers is less than one percent (DHHS 1986; Watts 1990). As a result, nicotine and cotinine levels are highly specific to tobacco smoke exposure.

Tar and nicotine yields are well correlated for most current brands of cigarettes (DHHS 1981). Therefore, nicotine or cotinine levels can be used to estimate the amount of particulate matter ("tar") absorbed and retained by a smoker. Cotinine is the preferred measure of nicotine absorption because of its longer 20-30 hour half-life in the blood (Benowitz 1983; IARC 1986). Cotinine is also less influenced by active smoking or environmental tobacco smoke than salivary nicotine levels.

Salivary, blood, or urinary levels of cotinine can represent the average daily dose of particulate phase constituents absorbed. This is important because the bulk of the carcinogenic activity in tobacco smoke is in the particulate phase (IARC 1986). Cotinine may also be useful for determining the net effect of changes that have opposing effects on smoke absorption, for example a cigarette with a lower tar and nicotine yield that is inhaled more deeply.

Thiocyanate

Hydrogen cyanide is found in the gas phase of cigarette smoke and is metabolized to thiocyanate by the body. Thiocyanate can be measured in plasma, urine, and saliva (IARC 1986). Although thiocyanate has an extremely long half-life (approximately 2 weeks), there are a number of foods that influence thiocyanate levels, including leafy vegetables and nuts. Dietary sources make the independent contribution of tobacco smoke to the thiocyanate level difficult to determine. Therefore, thiocyanate is an imprecise and poorly reproducible quantitative marker for smoke absorption.

Other Compounds

A number of other constituents of tobacco smoke, or metabolic products of tobacco smoke constituents, have been used as measures of smoke absorption including N-nitrosoproline and thioethers (IARC 1986). However, none of these measures appear to offer any advantages in estimating smoke absorption over the three measures described above.

TESTING FOR TOXIC EFFECTS OF THE SMOKE ABSORBED BY THE SMOKER

A number of short-term toxic effects of human tobacco smoke inhalation and retention can be measured. Some are postulated to be early or intermediate steps leading toward seriously adverse health effects (Chapter A). These effects include reduced serum high-density to low-density lipoprotein ratios, increased platelet aggregation, acute bronchial reactivity to smoke inhalation, small airway dysfunction in the lung, and greater mutagenicity of the urine. Measurement of these effects might possibly define the degree of damage being done by the smoke in a way that would integrate the effects of dose of exposure and biologic susceptibility. In addition, they might also detect pathophysiological toxicities that would not be evident from chemical analyses or markers of whole smoke exposure.

The limitations of these tests include their variability of measurement, the relatively long period of time required for smoking behavior to produce measurable change, and the degree of scientific uncertainty concerning their role in the pathophysiology of smoking-related disease. In addition, the general population varies widely in the levels of most of these effects. Due to the large variation and possibly small relative changes, a large group of volunteers may be needed to attain statistical significance.

COMPARISON OF LOW IGNITION-POTENTIAL CIGARETTES

The ideal comparison "standard" for the low ignition-potential candidate brands would be existing cigarette brands tested under the same protocol. This would allow comparison of the toxicity of the candidate brands with the range of toxicities for the currently marketed brands.

One approach is to compare the risk estimates for the candidate brand to the range of risk estimates for brands currently on the market. For example, low ignition-potential cigarettes with risk estimates that are within the range of those for currently marketed brands might be assumed to be no more hazardous than current brands of cigarettes. Risk estimates for current brands of cigarettes would be obtained by performing the same tests used on the low ignition-potential candidates. This approach avoids the problem of defining an acceptable standard for low ignition-potential cigarettes.

A variant of this approach would be to require no increase in the sales-weighted average risk for all of a given cigarette manufacturer's products when a low ignition brand of cigarettes is marketed. This allows the manufacturer to adjust the mix of their brands to avoid an overall increase in risk. These

approaches assume that existing brands of cigarettes will be allowed in the market even though they may have higher ignition potential.

Another approach, which does not assume that existing brands will continue to be marketed, compares a candidate brand with the one it was intended to replace or duplicate in the marketplace or the one from which it was developed. This approach would ensure that a new cigarette brand would not increase the average risk of cigarette smoking. However, it may not always be possible to pair a candidate brand with an existing brand.

SOURCES OF INCREASED HAZARDS

The major measurable increases in the hazard of smoking low ignition-potential cigarettes compared to current cigarettes are likely to come from differences in the:

1. cigarette manufacturing process or tobacco used that lead to a greater total yield of mainstream or sidestream smoke,

2. pattern of smoking that lead to a greater total yield of mainstream or sidestream smoke,

3. chemical composition of mainstream and sidestream smoke produced that lead to more toxic or carcinogenic smoke,

4. pattern of smoking that lead to greater absorption of smoke, and

5. additives to the cigarette that increase the toxicity or add new toxicities to the smoke.

Changes in toxicity could be evaluated for each of the many different possible designs that could reduce the ignition potential of cigarettes. However, this approach would be both impractical and wasteful since the many different ignition-potential reducing strategies would generate a prohibitively large number of combinations to be tested. The vast majority of the combinations would also be unlikely to be used in commercial cigarettes. Thus, it is only necessary to examine those combinations of low-ignition-potential changes that cigarette manufacturers present as potential commercial brands.

RECOMMENDATIONS

Generation of information to assess the risks of low ignition-potential cigarettes is product research and toxicity testing, a responsibility most appropriately belonging to the

cigarette manufacturers. The data should be generated by the manufacturer when contemplating the introduction of a new brand or replacement of an existing one. In addition, a new low ignition-potential cigarette is likely to combine designs to reduce the ignition potential with those to improve the taste or other marketing characteristics of the cigarette. It is the combination of all changes in a low ignition-potential cigarette, not just those that reduce the ignition potential, that determine its relative toxicity. Therefore, these cigarettes will need to be examined on a brand-by-brand (product performance) basis rather than on a manufacturing change-by-change (product design) basis. The following data should be collected on a candidate cigarette brand:

1. Smoking topography
   The number of cigarettes smoked per day, puff volume, puff duration, puff interval, maximal puff inspiratory pressure and flow, and number of puffs per cigarette should be measured in an experimental group of smokers of the brand after they have acclimatized to smoking the low ignition-potential brand.

2. Smoke yield and composition
   The range of mainstream and sidestream concentrations of the compounds listed in Table 3 of Chapter D should be assessed for smoke produced by machine smoking the brand using a range of smoking topographies that correspond to those observed for that specific brand.

3. Additives
   A complete list of additives and the concentrations used, as well as their likely pyrolysis products, should be disclosed for each brand. Confidential Business Information status may be requested to protect proprietary information.

4. Inhalation and retention of smoke
   The change in CO level that results from smoking a single cigarette of the brand should be measured in acclimitized smokers as a marker for acute exposure. Cotinine levels should be measured in acclimatized smokers of the brand as a marker for average daily exposure.

5. Toxicity of the smoke produced
   The toxicity of the mainstream and sidestream smokes produced by machine smoking each brand of cigarettes should be evaluated using the approaches described in Chapters E and F of this report.

Testing Sequence

The sequence of testing for the evaluation of toxicity and carcinogenicity of low-ignition-potential brands of cigarettes should include the following stages for mainstream and sidestream smokes:

I. Initial Evaluation

1. Machine testing

Initial evaluation of each candidate brand before testing in humans, should include analyses of CO, nicotine, tar, and each of the other constituents of tobacco smoke listed in Table 3 in Chapter D using the FTC method (Chapter B) to generate the smoke.

If the smoke produced using the FTC method did not yield substantially greater amounts of the compounds listed in Table 3 of Chapter D, then testing would proceed using a range of smoking topographies observed for current brands of cigarettes. Topographies reported by several studies for current cigarettes (Table 1) are graphically presented in a puff volume / puff duration matrix (Table 2 of this chapter). The central cells in this matrix are the most commonly reported values from the studies in table 1 and the values for the FTC method. Tar, nicotine and CO yields should be measured under the smoking conditions specified by each shaded and unshaded cell (15 ml/1 sec through 90 ml/3 sec) to reflect the range of observed human topographies.

The toxic chemical constituents in Table 3 of Chapter D should then be analyzed using smoke generated according to the topographies indicated by the shaded cells of Table 2 in this chapter (except for the FTC protocol, which has already been conducted). These represent the most common and the extremes of reported topographies. The reason for testing under conditions reflecting the extremes is to examine the effects on yield of using rapid and slow puffing, and large and small puff volumes. It is under these conditions that unexpected changes in smoke yield and composition are most likely to occur. The smoke consitutents should also be analyzed for any cell that resulted in a tar level that was substantially higher than the average for the entire matrix. Smoke constituents should be reported as a fraction of the tar generated, for example nicotine/g of tar.

If the ranges of the constituents generated using the smoking profiles defined by this matrix is within or below the range for existing commercial brands of cigarettes, the candidate cigarette may proceed to the biological testing stage. If one or more of the constituents exceeded the range for current brands of cigarettes, a risk benefit analysis which includes the other constituents and the reduction in ignition potential may decide whether to reject the cigarette or to proceed with biological testing.

2. Biological testing

Biological testing, described in Chapters E and F, can be conducted utilizing mainstream and sidestream smoke generated using the FTC method and the topographies specified by any cell in the matrix in Table 2 where the ratio of toxic constituents to tar content is statistically significantly greater than the mean value for all of the tested cells (see section of machine testing above). The results from the tests of the low ignition brands can be compared with the range of the results available from testing of existing commercial brands. An increase in the toxicity of the candidate brand over the range of results for currently marketed brands may be considered an increase in the health hazard attributable to the candidate brand.

3. Additives

A complete list of the levels of additives in the candidate cigarette brand and the probable identities of associated pyrolysis products should be reported. If the toxicity data on these additives and pyrolysis products are not available, additional toxicity testing should be performed.

II. Characterization of topography in humans

Topography studies in humans may be conducted when the initial evaluation of the candidate brand indicates it may be no more hazardous than current commercial brands of cigarettes (considering both disease risks and ignition potential). Human studies would define the actual topography of smoking that occurs with a new low ignition-potential cigarette. It would also collect data on the associated markers of smoke absorption, which are needed for determination of human risk.

Volunteers should be selected to represent a balanced gender, socioeconomic, and ethnic/racial distribution. Additionally, the group should be selected to equally represent smokers who smoke the full range of the number of cigarettes per day of cigarette brands with nicotine yields (FTC method) in the top, middle and lowest thirds of the current brands of cigarettes by market share. In order to be confident that there is adequate representation of each of these factors in the study, at least 200 smokers should be selected for each study group.

Data collected before and after switching to the candidate brand include smoking topography, cigarettes smoked per day, urinary cotinine, and CO levels in the breath before and after smoking a candidate cigarette. The volunteers would be allowed to acclimatize to the candidate brand for two to three weeks. Mean values of the markers of smoke absorption that exceed the range reported for current cigarettes or increases in mean levels

that result from switching to the prototype brand would be evaluated for toxicity concerns.

The range of smoking topography observed in the groups should then be compared to the matrix in Table 2 of this chapter. If the results are significantly above or below the ranges in the table, then chemical analysis and toxicity testing must be repeated using the topographies that were outside the matrix.

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Table 1

Smoking Topography		Number of Subjects	Cigarettes Per Day	Puff Volume (ml)	Puff Duration (sec)	Peak Flow (ml/sec)	Puffs Per Cigarettes	Inter-Puff Interval (sec)	Cigarette Duration (sec)	Inhalation Volume (ml)
Rawbone (1978) Low Tar	mean s.e.m.	5			1.74 ± 0.28		9.8 ± 1.07	43.6 ± 5.430		
Rawbone (1978) Middle Tar	mean s.e.m.	7			1.92 ± 0.205		10.9 ± 0.77	38.8 ± 4.873		
Jarvik (1978)	mean	9	18.5				9.7			
Guillerm (1978)	mean range	8		38.5 15-80	1.85 0.8-2.9	35 15-50	12 5-16	40.7 23-115	390	452
Ashton (1978)	mean	14	10-25 (range)		1.47			24.0		
Schultz (1978) 1971 Group	mean	100			1.41		10.5	50.3		
Schultz (1978) 1974 Group	mean	218			1.34		11.8	41.5		
Moody (1980)	mean s.d.	517	20.7 ± 12.5	43.5 ± 21.9	2.12 ± 0.88		9.26 ± 3.79	25.8 ± 17.1	[1]232.2 ± 103.8	
Russell (1980b)	mean	10	26.7				10.7			
Henningfield (1981)	mean 95% c.i.	8	26 [2]15-35		.99 [2]0.70-1.19		9.9 ± 0.30	39.4 ± 1.54	351 ± 9.0	
Stepney (1981)	mean s.d.	19	[3]18.4 ± 8.1				12.9		400	485
Battig (1982) [4]male	mean s.e.m.	67	22.3 ± 9.0	43.7 ± 14.0	2.28 ± 0.5		12.1 ± 4.2	24.8 ± 10.7		527 ± 196.1
Battig (1982) [4]Female	mean s.e.m.	43	20.9 ± 8.6	37.1 ± 10.1	1.99 ± 0.42		13.0 ± 4.5	24.7 ± 11.6		481 ± 208.2
Epstein (1982) Male	mean s.e.m.	33	20.1 ± 1.9		[5]33.1 ± 1.61		12.7 ± 0.6			278.5 ± 20.8
Epstein (1982) Female	mean s.e.m.	30	14.9 ± 1.5		[5]27.84 ± 1.55		13.3 ± 0.6			263.0 ± 22.3
Russell (1982) Usual Brand	mean s.d.	12	23.8 ± 8.1	39.8	2.3		14.7	25.6	[1]324	547

		Number of Subjects (ml)	Cigarettes Per Day	Puff Volume (ml)	Puff Duration (sec)	Peak Flow (ml/sec)	Puffs Per Cigarettes	Inter-Puff Interval (sec)	Cigarette Duration (sec)	Inhalation Volume (ml)
Gritz (1983) Baseline	mean s.d.	8	27.5 ± 10.4	65.6 ± 27.6	2.2 ± 0.5	48.1 ± 23.3	9.3 ± 2.1	47.2 ± 16.2		600 ± 269.8
[6]Ossip-Klein (1983)	mean s.d.	9	30.0 ± 14.1		1.4 ± 0.1		7.2 ± 1.5		[1]378 ± 108	
[7]Ossip-Klein (1983)	mean s.d.	9	29.2 ± 11.1		1.7 ± 0.6		10.3 ± 4.6		[1]402 ± 84	
[8]Ossip-Klein (1983)	mean s.d.	6	45.0 ± 8.4		1.3 ± 0.5		13.6 ± 6.7		[1]510 ± 90	
Gust (1983)	mean	8	16	43.7	1.6		9.0	48.4	393	
Adams (1983)	mean s.d.	10		44.2 ± 12.7	1.88 ± 0.91			25.9 ± 14.5		614 ± 358
Nil (1984) [9]Group 1	mean s.e.m.	10	19.5 ± 6.8	41.1 ± 13.6	1.67 ± 0.50	41.1 ± 13.7	14.8 ± 3.8	25.9 ± 14.8		619 ± 329.3
Nil (1984) [7]Group 2	mean s.e.m.	10	20.5 ± 11.3	39.5 ± 8.9	1.57 ± 0.41	39.5 ± 8.9	14.7 ± 4.7	25.7 ± 11.9		579 ± 244.8
McBride (1984) Own Brand	mean s.d.	9		42.1 ± 7.5	2.14 ± 0.56		15.7 ± 3.8	25.3 ± 8.4	352 ± 69	
Medici (1985) Healthy Smokers	mean s e.m.	17		43.1 ± 3.8	2.2 ± 0.2	31.4 ± 1.6	13.7 ± 0.7	18.7 ± 2.6		
Burling (1985) Low CO	mean s d	12	21.4 ± 11.5		1.65 ± 0.3		12.6 ± 3.7	26.1 ± 3.7	316.8 ± 80	
Burling (1985) High CO	mean s d	12	19.4 ± 7.6		1.65 ± 0.3		11.0 ± 2.2	30.4 ± 8.5	342.5 ± 85.0	
Nil, Buzzi, Battig (1986) Male	mean s d.	69	25.2 ± 14.7	42.3 ± 14.5	2.18 ± 0.71	34.8 ± 10.6	12.6 ± 4.6	22.9 ± 12.8		511.6 ± 220.1
Nil, Buzzi, Battig (1986) Female	mean s.d.	48	24.2 ± 14.1	41.4 ± 13.3	2.02 ± 0.52	36.2 ± 8.4		21.7 ± 10.7		509.8 ± 224.4
Hughes (1986) Type A	mean s.e.m.	19	23.7 ± 2.0		1.58 ± 0.1		11.9 ± 0.8			
Hughes (1986) Type B	mean s.e.m.	27	21.0 ± 1.4		1.56 ± 0.1		10.4 ± 0.4			
Bridges (1986) Group 1	mean s.e.m.	5	[7]19.4 ± 5.4	85.4 ± 10.6			13.2 ± 2.8			

		Number of Subjects (ml)	Cigarettes Per Day	Puff Volume (ml)	Puff Duration (sec)	Peak Flow (ml/sec)	Puffs Per Cigarettes	Inter-Puff Interval (sec)	Cigarette Duration (sec)	Inhalation Volume (ml)
Bridges (1986) Group 2	mean s.e.m.	16	[9]23.1 ± 2.6	63.7 ± 8.0			11.4 ± 1.4			
Bridges (1986) Group 3	mean s.e.m.	22	[9]24.7 ± 6.6				11.1 ± 0.9			
Bridges (1986) Group 4	mean s.e.m.	65	[9]25.6 ± 1.6	52.2 ± 1.8			10.6 ± 0.5			
[10]Woodman (1986)	median range	9	22 15-30	47.9 32.0-59.0	1.6 1.1-1.3		13.0 8-19	18.0 6.7-36.6	255 127-376	639 284-1006
Nemeth-Coslett (1986a) Placebo	mean range	8	33 20-50				8.2	64	414	
Nemeth-Coslett (1986b) Placebo	mean ± s.e.m.	8	[2]30 (20-40)		1.4 ± 0.2			47 ± 6.4	362 ± 28.6	
Nil, Woodson, Battig (1986) High CO Absorbers	mean s.d.	19	32.3 ± 7.8	34.2 ± 10.8	2.2 ± 0.6	33.2 ± 13.0	14.6 ± 4.3	21.3 ± 9.5		485.2 ± 171.0
Nil, Woodson, Battig (1986) Low CO Absorbers	mean s.d.	20	19.7 ± 16.6	25.4 ± 8.1	1.9 ± 0.6	22.8 ± 6.4	15.0 ± 3.8	22.0 ± 7.7		372.6 ± 180.0
Nil, Woodson, Battig (1986) Nonselected Smokers	mean s.d.	132	28.5 ± 12.4	30.3 ± 11.2	2.2 ±0.7	27.6 ± 10.5	12.7 ± 5.3	28.0 ± 15.4		375.7 ± 210.4

1.Units changed from minutes to seconds

2.Range

3.Units changed from weeks to days

4.First cigarette smoked data

5.Total puff time

6.Experiment 1, naturalistic setting data

7.Experiment 2, naturalistic setting data

8.Experiment 3, naturalistic setting data

9.Number cigarettes in past 24 hours

10.Data from first visit

Table 2

Matrix for Machine Measurement of Tobacco Smoke Yield					
	Puff Volume (ml)				
Puff Duration (sec)	15	30	45	60	90
1					
2					
3					

Chapter D

Analysis of Toxic Smoke Constituents

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Contents

Introduction

The chemical analytical evaluation of several key constituents of cigarette smoke is a useful predictor of potential toxic and/or tumorigenic activity of the combustion products of cigarettes. This chapter describes the methodology for the determination of select known toxic and tumorigenic agents in tobacco smoke as well as the standardized analytical procedures that can be applied to the evaluation of the vapor phase and particulate matter of cigarette smoke.

Although sidestream smoke (SS) of cigarettes is a major contributor to environmental tobacco smoke, and the emission of SS constituents from prototype cigarettes may raise some health concerns, this chapter does not specifically address the overall systematic analytical elucidation of sidestream smoke composition, yet it highlights some compounds in SS that are relevant to health concerns.

The burning of cigarettes generates mainstream smoke (MS) during puff drawing and SS during smoldering between puffs. The physicochemical nature of these smoke types is dependent upon factors such as the type of tobacco, the temperatures prevailing during puff-drawing (860-900°C) and smoldering (500-650°C), the reducing atmosphere that is characteristic of the burning cone, and the physical design of the cigarette (e.g., length, diameter, filter tip, and type of cigarette paper). These different parameters also influence the ignition propensity of a cigarette.

The 400-500 mg of mainstream smoke that are freshly emerging from the mouthpiece of a cigarette are an aerosol that contains about $1x10^{10}$ particles per milliliter in the vapor phase (1). The range in diameter is 0.1-1.0 μm with a mean of about 0.2 μm. About 95% of the MS effluent of a nonfilter cigarette is comprised of 400-500 individual gaseous compounds with nitrogen, oxygen and carbon dioxide as major constituents. Until now, at least 3500 individual compounds have been identified in the particulate matter (Figure 1; 2, 3).

For chemical analysis, MS is arbitrarily separated into vapor phase and particulate phase. Individual compounds of which more than 50% appear in the vapor phase of fresh MS are considered volatile smoke constituents; all others are particulate phase components (Figure 1). Tables 1 and 2 list the major types of compounds identified and their estimated concentrations in the smoke of one nonfilter cigarette (4). All data were derived from machine-smoking of cigarettes under standardized laboratory conditions (5). [Machine smoking does not necessarily reflect the range of human smoking behavior characteristics (6-8, Topography chapter).]

Tables 1 and 2 do not contain information about the presence of agricultural chemicals and pesticides that originate from the residue of such compounds on the tobacco (9). These residues differ in respect to chemical nature, concentrations, and type of agricultural chemicals used in the various tobacco-growing countries, and they may vary from season to season (10). The list also lacks information on flavor additives, because the nature and composition of such agents remain trade secrets. One exception among flavor additives is menthol of which up to 500 ug/cigarette may be found in MS (11).

Tobacco is known to contain at least 30 metals (12). In general, less than 1% of the metals is transferred from the tobacco into the MS of a cigarette (13). The levels of these elements are very low and thus are not listed in Table 2. However, the formulation of cigarettes with lower ignition propensity may include metallic additives. Therefore, it may be advisable to consider the analysis of added metals if they are suspected of having biological significance.

I. Toxic Agents in Tobacco Smoke

Hundreds of studies have been concerned with the chemical nature and quantitative aspects of toxic and tumorigenic agents in the mainstream smoke of cigarettes. Table 3 lists those toxic agents that have been most extensively studied and are considered to be major contributors to the toxicity of the smoke (14-16). This listing is not complete; however, the concentrations of all of these compounds give sufficient indication of the overall toxicity and tumorigenicity of the MS of a particular cigarette relative to a control cigarette, or to other commercial cigarettes. The possible presence and effects of additives to the tobacco or paper and their corresponding combustion products in the MS should be considered. The determination of such additives and their combustion products in the MS and SS may require specific methods.

The yields of particulate matter in undiluted SS are 1.3-1.9 times higher than those in the MS of cigarettes. About 30-40 compounds have been identified and quantified in the particulate matter of SS. Nicotine levels in undiluted SS are 2-4 times higher than in MS. However, SS yields of aromatic amines exceed those in MS 20-to-30-fold. Undiluted SS also contains remarkably high levels of ammonia and of N-nitrosodimethylamine and N-nitrosopyrrolidine (Table 4). The greater release of the trace metals into SS might point toward increased releases of metallic additives into environmental smoke.

SS which is generated during smoldering of the cigarette, is the major contributor to environmental tobacco smoke (ETS) (or secondhand smoke). Minor contributions to ETS are made by vapors

diffusing through the cigarette paper, smoke escaping from the mouthpiece or burning cone, and exhaled smoke. Table 5 presents some of the data for toxic agents reported in indoor environments.

II. Smoking Conditions

The analysis of most of the individual smoke compounds requires 20 cigarettes or less. Cigarettes should be selected, prepared, and smoked individually by the standard smoking conditions established by the Federal Trade Commission in 1969 as modified in 1979 (22), described in Chapter B, unless human data (Chapter C) indicates otherwise. Additionally, cigarettes within ± 20 mg of the average weight of 200 cigarettes should be selected for analysis. In the case of filter cigarettes, draw resistance must be within ± 5% of the average of the weight-selected cigarettes (14). Quantitative assessment of most of the individual smoke compounds requires 20 cigarettes or even less.

When analyses require 20 cigarettes or less, piston-type smoking machines (Chapter B), such as the 20-channel Phipps and Bird smoker, should be employed (23). When the analysis requires more than 20 cigarettes, a constant-volume-constant-time smoking machine, such as the Borgwaldt-20 smoker with rotating head, may be preferred (Chapter B). [The latter is available in the US through International Planters Corp., Borgwaldt Division, P.O. Box 24505, Richmond VA, 23224; phone 804-230-0011]. The machines with rotating head are preferred because they allow flushing with nitrogen after each puff in procedures where avoidance of artifacts is important. They also require less space and are easy to operate. The determination of each smoke constituent should be completed in duplicate or triplicate.

III. Analysis of Individual Smoke Constituents

The undesirable effects of cigarette smoking relate to the exposure to toxic, ciliatoxic, tumor-initiating and tumor-promoting agents and to organ-specific carcinogens. The selection of key compounds in cigarette smoke that are to be determined analytically for the evaluation of the toxic and tumorigenic potential of cigarette smoke is guided by knowledge on the proven toxic and/or tumorigenic effects in bioassays (Tables 3 and 4). The extent to which these same agents are toxic and/or tumorigenic to humans is not always known but can be deduced from interpretation of their probable activities made by the International Agency for Cancer Research (IARC Monograph Series, Vols. 1-53; 1972-1991).

The carcinogenicities of tobacco and tobacco smoke in bioassays as well as the tobacco-related cancers in human epidemiologic studies are dose-related. Human risk assessment therefore depends not only on the relative biologic potency of individual or total tobacco carcinogens, but also on the quantitative aspects of exposure as these relate to personal smoking habits. Since smoking of cigarettes by machines mimics yet never completely duplicates the smoking patterns of individuals, the analytical evaluation of key components of tobacco smoke by standardized methods provides primarily valid comparisons of the relative toxic and tumorigenic potential of one cigarette brand over another.

It is proposed that MS should be analyzed for 14 parameters. These are listed below. The cited analytical methods are known to be reproducible within a given laboratory. However, only methods for total particulate matter ("tar"), carbon monoxide, and nicotine are standardized. Methods for these three were compared in collaborative studies between laboratories.

Measures should be taken to avoid artifacts that might occur during cigarette smoke analysis, e.g. aging of smoke resulting in the conversion of NO to NO_2, nitrosamine formation during trapping, degradation of carbonyl chemicals by secondary reactions, etc. Where practical, at least two methods are suggested for each parameter.

1. Total Particulate Matter (TPM-dry)

The major carcinogenic activity of tobacco smoke resides in the particulate matter. Therefore, the total yield of particulate matter in the smoke of a given cigarette is a key determinant of its carcinogenic potential. However, the vapor phase does contain additional toxins and tumorigenic agents which must be determined for a complete assessment of inherent risk.

Macro-methods of assessment, such as fluorescence of TPM solutions or elemental analysis are not recommended. Those methods are not sufficiently specific to be associated with the carcinogenic activity of TPM.

The method for TPM determination, including its moisture content should be based on the FTC-method (22), described in detail in Chapter B. Reproducibility of the data will be confirmed by including a University of Kentucky standard reference cigarette during the analyses.

2. pH of Mainstream Smoke

Nicotine, the major habituating agent and an important toxic compound in tobacco smoke (19, 24), is protonated in the MS of U.S. blended cigarettes and of cigarettes that are made entirely with bright tobacco, because the smoke of these cigarettes rarely exceeds pH 6.2 (18). Higher smoke pH increases the toxicity. At pH above 8.0, which occurs in smoke from cigarettes made entirely with black or burley tobacco, more unprotonated nicotine is present in the vapor phase. This tends to raise blood pressure far more rapidly and to a greater extent than does the protonated (bound) nicotine in the smoke (24, 25). Thus, the pH of the mainstream smoke should be measured.

Two methods are usually followed for determining smoke pH. The method developed by Sensabaugh and Cundiff (26) and applied by others (18) provides for measurement of the pH of individual puffs. In this case, single cigarettes are smoked by a piston-type machine. Each puff is led over a modified electrode; the latter is connected to a pH-meter (Beckman Model SS-2) and the signals are recorded by a strip-chart recorder (18). The test is run in triplicate to obtain representative values. The advantage of this method lies in its ability to record the pH of each puff from the first to the last puff. This method has been utilized primarily for research; it requires constant recalibration and maintenance.

Grob describes another method in which three cigarettes are machine-smoked under standard conditions through a 40-ml glass tube that is loosely filled with cotton (27). After the smoking, the cotton is washed with 40 ml CO_2-free distilled water and subsequently twice more with 10 ml water. The pH of the combined water extracts is measured with a pH meter resulting in an average value from all puffs of a cigarette.

The average pH of a cigarette can also be crudely measured by smoking a cigarette puff by puff through 2 interconnected impingers filled with 10 ml CO_2-free distilled water each (total free volume 40 ml). After 2 or 3 cigarettes have been smoked, a clearing puff is taken, the contents of the impingers are combined with 20 ml of water used for rinsing the impingers and, after filtration, the pH of the whole smoke is measured. To obtain representative pH values this test has to be done in triplicate.

A smoking machine-extractor device has been developed for large-scale determinations of the smoke pH of cigarettes. The device is reported to deliver reproducible, average pH values within 20 minutes (28).

3. Redox Potential of Cigarette Smoke

It has been demonstrated that the reducing property of cigarette smoke adversely affects the respiration of the mammalian cell. Specifically, the redox potential of the smoke of a given tobacco product is correlated with the degree of cytochrome C reduction (29, 30). It is known that the redox potential in each puff of cigarette smoke gradually declines from the 230-240 mV initially measured for air with the reference calomel electrode to the 140-160 mV in the smoke of the last puff. Smoking a cigarette through a glass fiber filter demonstrates that the gaseous phase is practically free of reducing agents and that the latter reside primarily in the particulate phase. Ammonia and hydroquinone do not contribute to the redox potential of cigarette smoke, and nicotine does so only to a minor extent. 1,4-Benzoquinone, naphthoquinones and anthraquinones appear to have a stabilizing effect on the redox potential of cigarette smoke (31, 32), thus diminishing the reducing effect of cigarette smoke on subcellular components of the mammalian cell.

To determine the redox potential of individual puffs, a piston-type smoking machine is connected with an apparatus containing a platinum (Pt) electrode which operates in conjunction with a reference calomel electrode (Figure 2; 32). The smoke is led over the Pt electrode only. Figure 3 shows the redox potential of 85-mm US blended non-filter cigarettes for puffs 1 through 8 (32).

Vapor Phase

4. Carbon Monoxide

The toxic burden of environmental carbon monoxide exposure is of special significance to cardiovascular health. The methodology for the determination of carbon monoxide in cigarette smoke is described in Chapter B.

5. Nitrogen Oxides (NO_x)

It is suspected that nitrogen oxides (NO_x) in cigarette smoke contribute to the development of pulmonary emphysema (30, 33) and the acceleration of platelet aggregation (34). They are potent inhibitors of thiol-dependent enzymes (35) and, at high concentration, they can induce bradycardia and arrhythmias (36). In addition, NO_x in cigarette smoke may prevent the activation of scavenger cells such as macrophages in the respiratory system. NO_x in cigarette smoke are also of concern because of their potential to nitrosate precursor amines and thus contribute to the formation of carcinogenic N-nitrosamines.

Cigarette smoke contains 3 forms of NO_x. These are nitrous oxide (N_2O; ≤ 1 µg/cigarette), nitrogen dioxide (NO_2; <10 µg/cigarette) and nitric oxide (NO; 6-600 µg/cigarette). The fresh smoke of a US non-filter cigarette contains 200-300 µg NO, depending on the nitrate content of the tobacco, which represents the major precursor for the nitrogen oxides in smoke (37). It is important for the analytical chemist to realize that unaged MS contains hardly any NO_2, but only NO, and that the latter oxidizes quickly to NO_2 (half-life in smoke, 6-10 minutes; 38, 39). This is of major consequence since NO_2 is essential for the toxicity and the N-nitrosamine formation in cigarette smoke (section III-9).

Several methods have been employed for determining NO_x in cigarette smoke. These include the widely used colorimetry (40-42), gas chromatography (43), nitrate ion electrode (44), infrared (39), and chemiluminescence (45, 46).

The colorimetric method is based on the Saltzman procedure (41). It involves the Griess reagent which reacts only with NO_2. For cigarette smoke analysis, NO must first undergo oxidation to NO_2. Sloan and Morie have discussed the shortcomings of the Saltzman method for the analysis of NO_x in cigarette smoke in great detail (44).

The preferred method for the determination of NO_x in cigarette smoke is by chemiluminescence. In this technique nitric oxide is measured by photoelectric amplification the chemiluminescent reaction of NO with ozone. NO_x ($NO+NO_2$) are measured by photoelectric amplification of the chemiluminescent reaction of NO_x and atomic oxygen, which is derived from thermal decomposition of O_3.

For chemilumniscent analysis, cigarettes are selected by weight and draw resistance and are smoked by a piston-type smoking machine through a Cambridge filter. The gas phase of each individual puff is directed through a gas sampling valve. An aliquot of the puff is injected into a GC column filled with a 100/200 mesh porous polymer (Chromosorb 104). The column temperature is set at 45°C. Argon, the carrier gas, is adjusted to a flow rate of 15 ml/min. The column exit is connected with a chemiluminescence detector (Figure 4). For each NO_x analysis, 4 cigarettes have to be smoked individually; this results in an experimental deviation for commercial US blended cigarettes of ±6% (detection limit = 0.5-1.0 µg NO per puff). The British Tobacco Research Council, London, refined the chemiluminescence method for NO_x in cigarette smoke and adopted it as a standard method (47).

6. Hydrogen Cyanide

Hydrogen cyanide (HCN) is an inhibitor of several respiratory enzymes; as such it can influence cellular metabolism in the myocardial and arterial wall. As a major ciliatoxic agent in cigarette smoke HCN greatly inhibits the clearance of tar components from the respiratory tract (19). Nitrate is a major precursor for HCN in the smoke (48), even though tobacco proteins are also precursors for HCN in smoke (49).

The methods developed for HCN analysis in cigarette smoke include ion-selective electrode titration, gas chromatography and coulometric methods (50). With one exception all methods measure cyanogen [$(CN)_2$] as part of HCN. However, $(CN)_2$ in the smoke of a cigarette amounts to less than 2.5% of the total HCN (51). To separate HCN from $(CN)_2$, the smoke of individual puffs of the cigarette is directed through a Cambridge filter and subsequently through gas wash bottles containing 100 ml 0.1N NaOH. When loaded with the smoke of 1 cigarette, the Cambridge filter is extracted with 100 ml 0.1N NaOH, washed, and combined with the solution from the gas wash bottles (total volume including washings 250 ml). A 1-ml aliquot is pipetted into a mixture of 2 ml 1.0 M NaH_2PO_4 with 1 ml chloramine-T solution and 20 ml n-hexane; it is then thoroughly mixed. One μl of the n-hexane layer is analyzed by GLC with ^{63}Ni-EC detector (51). The cyanogen chloride, formed by the reaction of HCN with chloramine-T gives a distinct peak which is clearly separated in the GC. The relative standard deviation of this HCN method is less than 5%, the detection limit is 50 ng HCN per cigarette. (Modification of the method by smoking more than 1 cigarette can greatly increase the detection limit).

Coulometric analysis for hydrogen cyanide by the method of Sloan (50) is recommended. It requires machine-smoking of 2 or more cigarettes through a scrubber containing 50 ml 0.1 N NaOH and through a Cambridge filter. Aliquots of the "smoked" scrubber solution plus washings are transferred to a titration cell which contains pH 11 buffer solution and 0.001 M lead acetate solution. The generator electrodes are connected to a coulometer, and the indicator electrodes are connected to the terminals of a potentiometer. The output of the potentiometer is connected to a strip-chart recorder. HCN that is not retained in the scrubber solution but trapped by the Cambridge filter is extracted with 0.1 N NaOH and an aliquot is titrated coulometrically as discussed for the aqueous scrubber solution. The two sets of data are combined and compared with data obtained from the other more involved methods. The standard deviation for this simple and rapid method for HCN in cigarette smoke was less than 6% (50).

7. Volatile Hydrocarbons

The gaseous phase of cigarette smoke has been shown to contain about 20-25 alkanes, up to 20 alkenes, some alkynes such as acetylene, a number of dienes, especially 1,3-butadiene and isoprene, and in addition to benzene, up to 30 volatile aromatic hydrocarbons (2, 3, 59). Together these hydrocarbons constitute 0.5-1.0% of the weight of the total mainstream smoke effluent of a cigarette. Despite this, few of them have been discussed as possible contributors to the toxicity of the smoke.

The exceptions are 1,3-butadiene and benzene. In inhalation studies in mice and rats, 1,3-butadiene is carcinogenic; however, the International Agency for Research on Cancer considers "the evidence for carcinogenicity of 1,3-butadiene to humans as inadequate" (17). Benzene, on the other hand, is a recognized occupational carcinogen, which upon long-term exposure increases the risk of workers for various types of leukemia (17). Case control studies and large-scale prospective follow-up studies have shown an association between cigarette smoking and leukemia especially myeloid leukemia (52-54). There is a correlation in cigarette smokers between urinary cotinine, a major metabolite of nicotine, and urinary *trans,trans*-muconic acid, a metabolite of benzene (55).

Rapid advances in chemical-analytical instrumentation have led to capillary GC-MS methods which enable the investigator to determine quantitatively within minutes dozens of volatile components in the vapor phase of only a fraction of individual puffs of a cigarette (56-58). These instruments are primarily helpful for research, they require great expertise; the methods are time consuming and generally not suitable for routine analyses of toxic hydrocarbons in the gaseous phase of cigarette smoke.

The following GC-MSD method is suggested for routine analysis of 1,3-butadiene, isoprene, benzene, and toluene. The gas phase of individual puffs from freshly generated mainstream smoke of selected cigarettes (section II) is led through a 1.0 ml sample loop. Upon reaching atmospheric conditions the sample loop is switched in line with gas chromatographic (GC) columns, first passing through a 5 m x 0.53 mm HP-1 precolumn and then onto a 30 m x 0.25 mm DB-5 analytical column. The effluent of the analytical column is diluted by the carrier gas, helium 1:25, and the emerging peaks representing individual volatile smoke components are determined by mass selective detection (GC-MSD).

The advantage of GC-MSD is that volatiles that were masked by the GC peak of a hydrocarbon in a regular GC-MS trace can be eliminated by selective ion monitoring and thus allow accurate quantification. The disadvantage of this method lies in the fact, that the hydrocarbons to be assayed have to be determined

for each individual puff separately in order to avoid losses during aging of the smoke. This is especially so for 1,3-butadiene (conventional smoking of a cigarette requires in general 8-12 puffs or 7-11 minutes before an aliquot of all puffs can be analyzed). For routine analysis, especially when comparing different cigarettes, the seventh puff is chosen as a representative puff of the cigarette (59).

8. Aldehydes

Formaldehyde, acrolein, and acetaldehyde together with hydrogen cyanide are the major ciliatoxic agents in cigarette smoke and are known irritants to the mucous membranes of the upper respiratory system and to the eyes. Upon inhalation they clearly contribute to the inhibition of lung clearance mechanisms, thus allowing extrinsic particles, such as tobacco smoke particulates, to settle (21, 60). Formaldehyde and acetaldehyde are known animal carcinogens. The International Agency for Research on Cancer regards formaldehyde as "probably carcinogenic to humans", acetaldehyde as "possibly carcinogenic to humans" and acrolein as "not classifiable as to its carcinogenicity to humans" (17).

Formaldehyde is determined by smoking 2 cigarettes individually through an 800-ml Kjeldahl flask containing a trap with 200 ml of saturated solution of 2,4-dinitrophenylhydrazine (DNPH) in 0.2 N HCl. After the smoking, the trapping solutions are extracted repeatedly with chloroform and triphenylene is added as an internal standard. The combined chloroform solutions are washed twice with 2 N HCl, then twice with water, and are then concentrated under a stream of nitrogen. The dried residue is dissolved in 5 ml methylene chloride. Aliquots are injected into a HPLC system which is described in great detail (61). The recovery rate is reported as better than 90% and the reproducibility better than 5%.

Acetaldehyde, acrolein, and propionaldehyde may be analyzed by the method of Manning (62) at Oak Ridge National Laboratory. The cigarettes are individually smoked and the volatile aldehydes are trapped by reacting them with DNPH in 2 N HCl solution. The concentrates of the 2,4-dinitrophenylhydrazones of the aldehydes are separated and analyzed by reverse phase HPLC with the absorbance detector at 365 nm. The detection limit is 10 μg aldehyde/cigarette, the relative standard deviation is about 12%.

9. Volatile N-Nitrosamines (VNA)

Volatile N-nitrosamines in cigarette smoke originate from the tobacco by transfer into the smoke, and from thermal degradation of nitrosamino acids, as well as from pyrosynthesis during smoking. For example, during tobacco processing proline is nitrosated to N-nitrosoproline (NPRO); its yield in the

tobacco is greatly influenced by the processing of the tobacco and by its nitrate concentration. During smoking NPRO gives rise to some N-nitrosopyrrolidine (NPYR) in MS (0.1-1%) and to much higher yields of NPYR in SS (20). Model studies have shown that volatile secondary amines can be nitrosated to nitrosamines in the vapor phase, however, the yields are low because freshly generated smoke contains primarily NO and only traces of NO_2. The latter is essential since the nitrosating agent for the formation of nitrosamines is N_2O_3 (64).

All 8 VNA identified in cigarette smoke (Figure 5) are organ-specific carcinogens in animals (65). For routine analyses usually only the 3 major VNA in cigarette smoke are quantitated. These are N-nitrosodimethylamine (NDMA; 0-75 ng/cigarette), N-nitrosodiethylamine (NDEA; 0-5 ng/cigarette) and N-nitrosopyrrolidine (5-40 ng/cigarette; 20).

The MS analysis requires 3 times 10-20 cigarettes without filter tips or 3 times 20-40 filter cigarettes; these have to be smoked individually under standard laboratory conditions (section II). [Cellulose acetate filter tips selectively remove VNA by 70% and more; thus, the VNA analysis in the smoke of filter cigarettes requires 20 cigarettes] (63, 66). The MS is led through a gas wash bottle containing 100 ml citrate-phosphate buffer, pH 4.5, with 20 mM ascorbic acid and an internal standard ([^{14}C]NDMA or N-nitrosodipropylamine). A Cambridge filter treated with a solution of ascorbic acid, is placed between gas wash bottle and smoking machine (after smoking 10 cigarettes the loaded Cambridge filter is replaced). The loaded Cambridge filter is thoroughly washed with dichloromethane (CH_2Cl_2) and the washings are filtered. The buffer solution in the gas wash bottle is extracted 4 times with 100 ml CH_2Cl_2, all organic-extracts are washed with 2 N NaOH (to remove interfering nitroalkanes), dried (Na_2SO_4) and concentrated to 5 ml. The concentrate is chromatographed on 65 g basic alumina (Woelm, activity II-III). The VNA are eluted from the column with 200 ml CH_2Cl_2, vacuum concentrated to about 1-2 ml and measured by GC-thermal energy analysis (63). The recovery rate is better than 70%. The detection limit is 0.05 ng NDMA per injection; the deviation coefficient for NDMA and NPYR is ± 5%; for NDEA it is up to ± 10%

Particulate Matter

10. Nicotine

The standard FTC-method for nicotine, the main pharmacologic agent in cigarette smoke (22), is described in Chapter B.

11. Phenols

More than 40 semivolatile phenols have been identified in cigarette smoke (3). The major precursors for these phenols in tobacco smoke are glucose, polysaccharides, pectins, rutin and other polyphenols. Minor amounts of semivolatile phenols that were formed in the tobacco during processing transfer into the smoke (14). It has been reported that the nonfilter 85-mm cigarette, made entirely from bright tobacco, delivered in the mainstream smoke 95 μg phenol, one made from Turkish tobacco yielded 120 μg, and from Maryland tobacco 60 μg; a burley tobacco cigarette produced 43 μg, and a US blend delivered 100 μg phenol (14). These findings indicate that the type of tobacco plays a major role in the yields of volatile phenols in cigarette smoke.

The volatile phenols contribute significantly to the tumor promoting activity of cigarette tar (14, 67, 68) and are active as ciliatoxic agents (21). The latter effect is one of inhibition or temporary paralysis of the mucus clearance that is normally provided by the ciliated epithelium of the respiratory tract. Ciliastasis allows foreign particles to remain in the respiratory tract where they can exert their particular activity or allow other agents to impair physiologic or biochemical functions.

The preferred analytical method for volatile phenols is gas chromatography of the weakly acidic portion of cigarette smoke condensate (69-71). Twenty to 40 cigarettes are smoked individually through a gas wash flask containing 2N NaOH with an internal standard (e.g. 2-chlorophenol, [^{14}C]phenol) and a Cambridge filter. The "loaded" Cambridge filter is extracted with 2N NaOH, filtered, combined with the NaOH solution from the gas wash bottle and the washings. This combined NaOH solution is extracted 3 times with ether to liberate the phenol concentrate obtained by solvent extractions without the need for concentrating by water steam distillation (72).

Cellulose acetate filter types, and especially those with specific plasticizers and with perforated filter tips reduce volatile phenols highly selectively (up to 85%); therefore, in some cases more than 40 or even 60 cigarettes are required for each analysis.

12. Catechols

The most abundant phenolic component in cigarette smoke is catechol (1,2-dihydroxybenzene; 80-400 μg/cigarette). Although 1 g of processed tobacco contains microgram levels of catechol, most of the catechol in the smoke is formed during the burning of tobacco from cellulose, monosaccharides, chlorogenic acid and pectins as precursors (73). Cigarette smoke also contains small

amounts of alkylated catechols including 3-methylcatechol (<20 µg/cigarette), 4-methylcatechol (~20 µg/cigarette) and 4-ethylcatechol (<25 µg/cigarette; 74).

Catechol is not a carcinogen but a very effective cocarcinogen in tobacco smoke. Upon co-application with benzo(a)pyrene or with other carcinogenic polynuclear aromatic hydrocarbons (PAH), it greatly enhances the carcinogenic activity of these agents (75, 76).

Two methods are primarily employed in the analysis of catechol, 1) enrichment of catechol from tar by distribution between solvent pairs, followed by a spectrophotometric method (77), and 2) enrichment of the catechols from the acidic fraction of cigarette tar by extraction with boric acid, followed by GLC (74). Both methods are simple, require only 20-40 cigarettes per analysis and are reproducible, when an internal standard is used (e.g. [^{14}C]catechol), within ± 6%.

Schlotzhauer (72) enriched the dihydroxybenzenes, catechols, resorcinols and hydroquinones from the weakly acidic fraction by gel filtration chromatography and analyzed the catechol concentrate by GC-MS. This method found catechol and six alkylcatechols, as well as other dihydroxybenzenes. It is used for the profile analysis of these types of chemicals in cigarette smoke.

13. Polynuclear Aromatic Hydrocarbons (PAH)

Inhalation studies with laboratory animals have demonstrated that the particulate matter of tobacco smoke induces malignant tumors of the respiratory tract, most notably in the larynx of the Syrian golden hamster (16, 79, 80). The particulate phase is much more carcinogenic than the gas phase (79). Fractions and subfractions of the particulate matter have been extensively assayed for tumorigenicity on rabbit skin and on mouse skin. It has been clearly demonstrated that the most tumorigenic fractions in these assays are those with highly concentrated PAH (14, 68).

However, the carcinogenic activity of cigarette smoke particulates cannot be explained by the presence of carcinogenic PAH alone. When PAH concentrates of the neutral fraction (<1% of whole tar) are combined with the tumor-promoting weakly acidic fraction, which by itself does not induce tumors, the tumor yield on mouse skin reaches 70-90% of the carcinogenic activity observed with the whole tar (68, 81). Thus, the PAH serve as tumor initiators. To date about 80-100 PAHs have been identified in cigarette smoke (most <10 ng cigarette). A PAH concentrate of the neutral fraction of cigarette smoke condensate was the only portion that induced squamous tumors in the lung of rats upon intratracheal instillation (82). Benzo(a)pyrene (BaP), a major

carcinogenic PAH in smoke (20-40 ng/cigarette), induced tumors in the hamster lung upon inhalation (83).

The International Agency for Research on Cancer regards 11 PAH and 3 nitrogen-containing PAH (aza-arenes) as established animal carcinogens. BaP, benz(a)anthracene and dibenz(a,h)anthracene are rated as "probably carcinogenic to humans" (Fig. 6; 17).

A great many studies were concerned with the analysis of PAH in cigarette smoke (14, 84). Often the PAH are enriched by distribution of cigarette tar between solvent pairs such as methanol-water (4:1) and cyclohexane followed by a second partition between cyclohexane and nitromethane which leads to a 10-fold enrichment of the PAHs (81). The PAHs are further concentrated by column chromatography followed by paper chromatography, or TLC; the individual PAH are then identified and quantitated by UV-spectrophotometry (85).

During the last 2 decades the final step of PAH analysis relies on capillary GC (86). Using an internal standard (e.g. [^{14}C] BaP) the recovery is better than 70%. The reproducibility for the major PAH (>5 ng/cigarette) is ± 8% with at least 100 cigarettes. Several PAH profile studies of the MS of nonfilter cigarettes showed the highest PAH yields for the smoke of cigarettes made entirely with bright tobacco (BaP= 35-53 ng/cigarette) and lowest PAH yields for cigarettes made entirely with burley tobacco (BaP= 20-24 ng/cigarette; 14, 18).

Most of the carcinogenic PAH in cigarette smoke (>90%) are pyrosynthesized via highly reactive C,H-radicals (14, 84) which result from thermal degradation of nonvolatile organic tobacco components. Since BaP is one of the most abundant carcinogenic PAH in cigarette smoke and its mechanism of formation is similar to the pyrosynthesis of other PAH, BaP is often quantitated as a monitor for the entire class of compounds in the smoke of a given cigarette. For this purpose, several quick methods for the analysis of BaP were developed recently (87, 88). Five to 10 cigarettes are smoked through a Cambridge filter assembly. One ml of cyclohexane for each 1 mg of TPM is used to agitate the mixture of the filter and tar for 1 hour. After filtration, the volume is reduced to 10 ml by rotary evaporation. Following refiltration through a 0.45 μm membrane filter, a 2 ml aliquot is chromatographed through a NH_2-Sep-Pak column, pre-conditioned with 10 ml hexane. The BaP fraction is eluted with 8 ml of hexane, evaporated to dryness, redissolved in 1 ml of acetonitrile and analyzed by reverse-phase HPLC using an isocratic solvent system (65% acetonitrile in water) and fluorescence detector (excitation = 289 nm; emission = 412 nm); benzo(e)pyrene served as internal standard (88).

14. Tobacco-Specific N-Nitrosamines (TSNA)

The TSNA are exclusively formed from nicotine and the minor *Nicotiana* alkaloids during tobacco processing and during smoking. So far 7 TSNA have been identified (Figure 7). Four of these are usually determined in cigarette smoke. These include the powerful organ-specific carcinogens N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). In mice, rats and hamsters these TSNA induce benign and malignant tumors of the lung, upper aerodigestive tract, pancreas and/or liver. The other two major TSNA are the weakly carcinogenic N'-nitrosoanabasine (NAB) and the non-carcinogenic N'-nitrosoanatabine (NAT; 89).

To determine the four major TSNA in cigarette mainstream smoke, 3 x 20 weight-selected cigarettes are smoked individually under standard laboratory conditions (section II; in the case of filter cigarettes selection must also be done according to average draw-resistance). The mainstream smoke is retained on a Cambridge filter (9.0 cm diameter) which is treated with a solution of ascorbic acid (90). The filter assembly is placed between the smoking machine and two gas wash bottles in line, each containing 60 ml distilled water to which 2 ml of 20% ammonium sulfamate solution in 3.6 N sulfuric acid is added, and also containing 0.5 μg [^{14}C]NNN as an internal standard (others have used N-nitrosodibenzylamine, N-nitrosopentylpicolylamine or 2-(methylnitrosamino)-1-(2-pyridyl)ethane; 91, 92). The total particulate matter trapped on the Cambridge filters is extracted twice with 100 ml ethyl acetate and the combined buffer solutions of the wash bottles are extracted with 3 times 100 ml ethyl acetate. The ethyl acetate extracts are dried (Na_2SO_4), concentrated to about 2 ml and chromatographed on 50 g basic alumina (Woelm, activity II to III) on a 2 x 20 cm column with 150 ml dichloromethane and a 4:1 mixture of dichloromethane: acetone (200 ml). The latter solvent mixture eluates the TSNA which are concentrated to 1-2 ml (recovery rate of [^{14}C]NNN= 75-85%).

The method of Adams (93) is recommended for the gas chromatography-thermal energy analysis (GC-TEA). The specific detector for NO-containing substances assures clear separation of NAB from NAT. The TSNA values are determined in a triplicate analysis within ± 7% (detection limit = 1 ng of a single TSNA per cigarette). Other analytical methods for TSNA were recently reported (20, 92).

IV. Postscript

It was the goal of this chapter to suggest analysis of those tobacco smoke parameters and smoke components that are considered

major contributors to the toxicity of cigarette smoke and are likely to be quantitatively affected by modifications that might reduce the ignition propensity of cigarettes. Athough this proposal is based on longstanding experience in the tobacco sciences and on a thorough study of the literature, it is not comprehensive for all toxicants which may occur in cigarette smoke. It was deemed important to keep the number of measurements practical.

For example, the determination of polonium-210 (^{210}Po) was not suggested, although the U.S. National Council on Radiation Protection and Measurement ascribed about 1% of the risk of lung cancer in long-term cigarette smokers to ^{210}Po (94). Polonium-210 in the smoke originates from the tobacco by transfer; a change in the make-up of a cigarette will not greatly alter the ^{210}Po concentration in the smoke (0.03-1.0 pCi ^{210}Po/cigarette; 14).

Similarly, analysis of nickel (0.1-0.6 μg/cigarette) or cadmium (<0.5 μg/cigarette) was not suggested even though the International Agency for Research on Cancer considers these elements as "carcinogenic to humans" and "probably carcinogenic to humans", respectively (17). As discussed earlier (section I), inorganic additives, including silicates, that are added to the tobacco or paper to reduce ignition propensity must be determined in the smoke in order to ascertain that the toxicity of the smoke is not increased.

Cigarette smoke also contains traces of a few known human carcinogens (17), such as 4-aminobiphenyl (2.4 ng/cigarette; 95), 2-naphthylamine (1.0 ng/cigarette; 95) and vinyl chloride (5-16 ng/cigarette; 96). Because these compounds are present in minute amounts, and analytical methods required for their determination are rather involved, we have not included the determination of these three chemicals in the overall analysis of toxic constituents. However, the analytical profiling of cigarette smoke can be extended to include these and/or any other agents, deemed to be of significance in respect to human health.

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Table 1 Major constituents of the vapor phase of the mainstream smoke of nonfilter cigarettes

Compound	Concentration/cigarette (% of total effluent)
Nitrogen	280 - 320 mg (56-64%)
Oxygen	50 - 70 mg (11-14%)
Carbon dioxide	45 - 65 mg (9-13%)
Carbon monoxide	14 - 23 mg (2.8-4.6%)
Water	7 - 12 mg (1.4-2.4%)
Argon	5 mg (1.0%)
Hydrogen	0.5 - 1.0 mg
Ammonia	10 - 130 μg
Nitrogen oxides (NOx)	100 - 600 μg
Hydrogen cyanide	400 - 500 μg
Hydrogen sulfide	20 - 90 μg
Methane	1.0 - 2.0 mg
Other volatile alkanes (20)[a]	1.0 - 1.6 mg[b]
Volatile alkenes (16)	0.4 - 0.5 mg
Isoprene	0.2 - 0.4 mg
Butadiene	25 - 40 μg
Acetylene	20 - 35 μg
Benzene	12 - 50 μg
Toluene	20 - 60 μg
Styrene	10 μg
Other vol. aromatic hydrocarbons (29)	15 - 30 μg
Formic acid	200 - 600 μg
Acetic acid	300 - 1700 μg
Propionic acid	100 - 300 μg
Methyl formate	20 - 30 μg
Other volatile acids (6)	5 - 10 μg[b]
Formaldehyde	20 - 100 μg
Acetaldehyde	400 -1400 μg
Acrolein	60 - 140 μg
Other volatile aldehydes (6)	80 - 140 μg
Acetone	100 - 650 μg
Other volatile ketones (3)	50 - 100 μg
Methanol	80 - 180 μg
Other volatile alcohols (7)	10 - 30 μg[b]
Acetonitrile	100 - 150 μg
Other volatile nitriles (10)	50 - 80 μg[b]
Furan	20 - 40 μg
Other volatile furans (4)	45 - 125 μg[b]
Pyridine	20 - 200 μg
Picolines (3)	15 - 80 μg
3-Vinylpyridine	10 - 30 μg
Other volatile pyridines (25)	20 - 50 μg[b]
Pyrrole	0.1 - 10 μg
Pyrrolidine	10 - 18 μg
N-Methylpyrrolidine	2.0 - 3.0 μg

Volatile pyrazines (18)	3.0 - 8.0 μg
Methylamine	4 - 10 μg
Other aliphatic amines (32)	3 - 10 μg

[a] Numbers in parentheses represent the individual compounds identified in a given group.

[b] Estimate

Table 2 Major constituents of the particulate matter of the mainstream smoke of nonfilter cigarettes

Compound	µg/Cigarette
Nicotine	1000 - 3000
Nornicotine	50 - 150
Anatabine	5 - 15
Anabasine	5 - 12
Other tobacco alkaloids (17)[a]	n.a.
Bipyridyls (4)	10 - 30
n-Hentriacotane [n-$C_{31}H_{64}$]	100
Total nonvolatile hydrocarbons (45)[c]	300 - 400[c]
Napthalene	2 - 4
Napthalenes (23)	3 - 6[c]
Phenanthrenes (7)	0.2 - 0.4[c]
Anthracenes (5)	0.05 - 0.1[c]
Fluorenes (7)	0.6 - 1.0[c]
Pyrenes (6)	0.3 - 0.5[c]
Fluoranthenes (5)	0.3 - 0.45[c]
Carcinogenic polynuclear aromatic hydrocarbons (11)[b]	0.1 - 0.25
Phenol	80 - 160
Other phenols (45)[c]	60 - 180[c]
Catechol	200 - 400
Other catechols (4)	100 - 200[c]
Other dihydroxybenzenes (10)	200 - 400[c]
Scopoletin	15 - 30
Other polyphenols (8)[c]	n.a.
Cyclotenes (10)[c]	40 - 70[c]
Quinones (7)	0.5
Solanesol	600 - 1000
Neophytadines (4)	200 - 350
Limonene	30 - 60
Other terpenes (200-250)[c]	n.a.
Palmitic acid	100 - 150
Stearic acid	50 - 75
Oleic acid	40 - 110
Linoleic acid	150 - 250
Linolenic acid	150 - 250
Lactic acid	60 - 80
Indole	10 - 15
Skatole	12 - 16
Other indoles (13)	n.a.
Quinolines (7)	2 - 4
Other aza-arenes (55)	n.a.
Benzofurans (4)	200 - 300
Other *O*-heterocyclic compounds (42)	n.a.
Stigmasterol	40 - 70
Sitosterol	30 - 40
Campesterol	20 - 30
Cholesterol	10 - 20

Anline	0.36
Toludines	0.23
Other aromatic amines (12)	0.25
Tobacco-specific N-nitrosamines (6)[b]	0.34 - 2.7
Glycerol	120

[a] Number in parentheses represent individual compounds identified.
[b] For details, see Figure 6.
[c] Estimate.
n.a. Not available.

Table 3. Compounds That Contribute To The Toxicity Of Cigarette Mainstream Smoke of US Nonfilter Cigarettes [a]

Compound	Yield/Cigarette Range	Toxic Effects
1. Total Particulate Matter (TPM, dry)	12-40 mg	Carcinogen[a]
2. pH		Influences nicotine toxicity[b]
3. Redox Potential		Influences toxicity of whole smoke[c]
Vapor Phase		
4. Carbon Monoxide	14-23 mg	Reacts with hemoglobin, inhibits O_2 transport[c]
5. Nitrogen Oxides(NO_x)	100-600 µg	Nitrosating agent[d], inhibitor of thiol dependent enzymes[c]
6. Hydrogen Cyanide	400-500 µg	Ciliatoxic[c]; inhibitor of respiratory enzymes[c]
7. Hydrocarbons		Suspected or known carcinogens[a]
benzene	12-50 µg	
1,3-butadiene	25-40 µg	
8. Aldehydes		Ciliatoxic[c], animal carcinogens[a]
formaldehyde	20-100 µg	
acrolein		
acetaldehyde	400-1400 µg	
9. Volatile N-Nitrosamines (VNA)		Strong animal carcinogens[a]
N-nitrosodimethylamine	13-65 ng	
N-Nitrosopyrrolidine	7-34 ng	
Particulate Matter		
10. Nicotine	1-3 mg	Associated with cardiovascular disease[c]
11. Phenols		Tumor promoters
phenol	80-160 µg	
other phenols	60-180 µg	
12. Catechol	200-400 µg	Major cocarcinogen
13. Polynuclear Aromatic Hydrocarbons		Major tumor initiators[a]
benzo(a)pyrene	20-60 ng	

14. Tobacco-Specific N-Nitrosamines		Strong organ-specific animal carcinogens[a]
NNN[d]	80-90 ng	
NNK[e]	60-470 ng	

[a] According to the International Agency for Cancer Research (17) TPM and benzene are human carcinogens, formaldehyde, benzo(a)pyrene and some other PAH as well as some volatile N-nitrosamines are probably carcinogenic to humans and acetaldehyde and the tobacco-specific N-nitrosamines are possibly carcinogenic to humans.

[b] Brunnemann and Hoffmann, 1974 (18).

[c] U.S. Surgeon General, 1983 (19).

[d] Brunnemann and Hoffmann, 1991 (20).

[e] Battista, 1976 (21).

[*] NNN - N'-Nitrosonornicotine
[*] NNK - 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone

Table 4. Some toxic and tumorigenic agents in undiluted cigarette sidestream smoke

Compound	Type of toxicity	Amount in sidestream smoke per cigarette	Sidestream: mainstream smoke ratio
Vapor phase			
Ammonia	T	50.0 - 130 μg	40 - 170
Carbon monoxide	T	26.8 - 61 mg	2-5 - 14.9
Carbonyl sulfide	T	2 - 3 μg	0.03 - 0.13
Benzene	C	240 - 290 μg	8 - 10
Formaldehyde	C	1500 μg	50
3-Vinylpyridine	SC	330 - 450 μg	24 - 34
Hydrogen cyanide	T	14 - 110 μg	0.06 - 0.4
Hydrazine	C	90 ng	3
Nitrogen oxides (NO_x)	T	500 - 2000 μg	3.7 - 12.8
N-Nitrosodimethylamine	C	200 - 1040 ng	20 - 130
N-Nitrosopyrrolidine	C	30 - 390 ng	6 - 120
Particulate phase			
Tar	C	14 - 30 mg	1.1 - 15.7
Nicotine	T	2.1 - 46 mg	1.3 - 21
Phenol	TP	70 - 250 μg	1.3 - 3.0
Catechol	CoC	58 - 290 μg	0.67 - 12.8
o-Toluidine	C	3 μg	18.7
2-Napthylamine	C	70 ng	39
4-Aminobiphenyl	C	140 ng	31
Benz[a]anthracene	C	40 - 200 ng	2 - 4
Benzo[a]pyrene	C	40 - 70 ng	2.5 - 20
Quinoline	C	15 - 20 μg	8 - 11
NNN	C	0.15 - 1.7 μg	0.5 - 5.0
NNK	C	0.2 - 1.4 μg	1.0 - 22
N-Nitrosodiethanolamine	C	43 ng	1.2
Cadmium	C	0.72 μg	7.2
Nickel	C	0.2 - 2.5 μg	13 - 30
Zinc	T	6.0 ng	6.7
Polonium-210	C	0.5 - 1.6 pCi	1.06 - 3.7

C, Carcinogenic; CoC, cocarcinogenic; SC, suspected carcinogen; T, toxic; TP, tumor promoter.

NNN - N'-Nitrosonornicotine
NNK - 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone

Table 5. *Some toxic and tumorigenic agents in indoor environments polluted by tobacco smoke*[a]

Pollutant	Location	Concentration/m^3
Nitric oxide	Workrooms	50 - 440 µg
	Restaurants	17 - 270 µg
	Bar	80 - 520 µg
	Cafeteria	2.5 - 48 µg
Nitrogen dioxide	Workrooms	68 - 410 µg
	Restaurants	40 - 190 µg
	Bar	2 - 116 µg
	Cafeteria	67 - 200 µg
Hydrogen cyanide	Living room	8 - 122 µg
Benzene	Public places	20 - 317 µg
Formaldehyde	Living room	23 - 50 µg
Acrolein	Public places	30 - 120 µg
Acetone	Public places	360 - 5800 µg
Phenols (volatile)	Coffee houses	7.4 - 11.5 ng
N-Nitrosodimethylamine	Restaurant, public place	0 - 240 ng
N-Nitrosodiethylamine	Restaurant, public place	0 - 200 ng
Nicotine	Public places	1 - 6 µg
	Restaurants	3 - 10 µg
	Workrooms	1- 13.8 µg
Benzo[a]pyrene	Restaurant, public place	3.3 - 23.4 ng
NNN	Public places	1.8- 22.8 pg
NNK	Public places	1.4- 29.3 pg

[a]References: Klus and Kuhn (97); IARC (17); US National Research Council (98); Klus et al. (99); Brunnemann (100).

NNN - N′-Nitrosonornicotine
NNK - 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone

Legends to Figures

1. Total Cigarette Smoke Compostition [% w/w] (2)

2. Approaches for Measuring Redox Potential of Cigarette Smoke (30)

3. Redox Potential of Puffs 1 to 8 of an 85 mm Plain U.S. Cigarette (30)

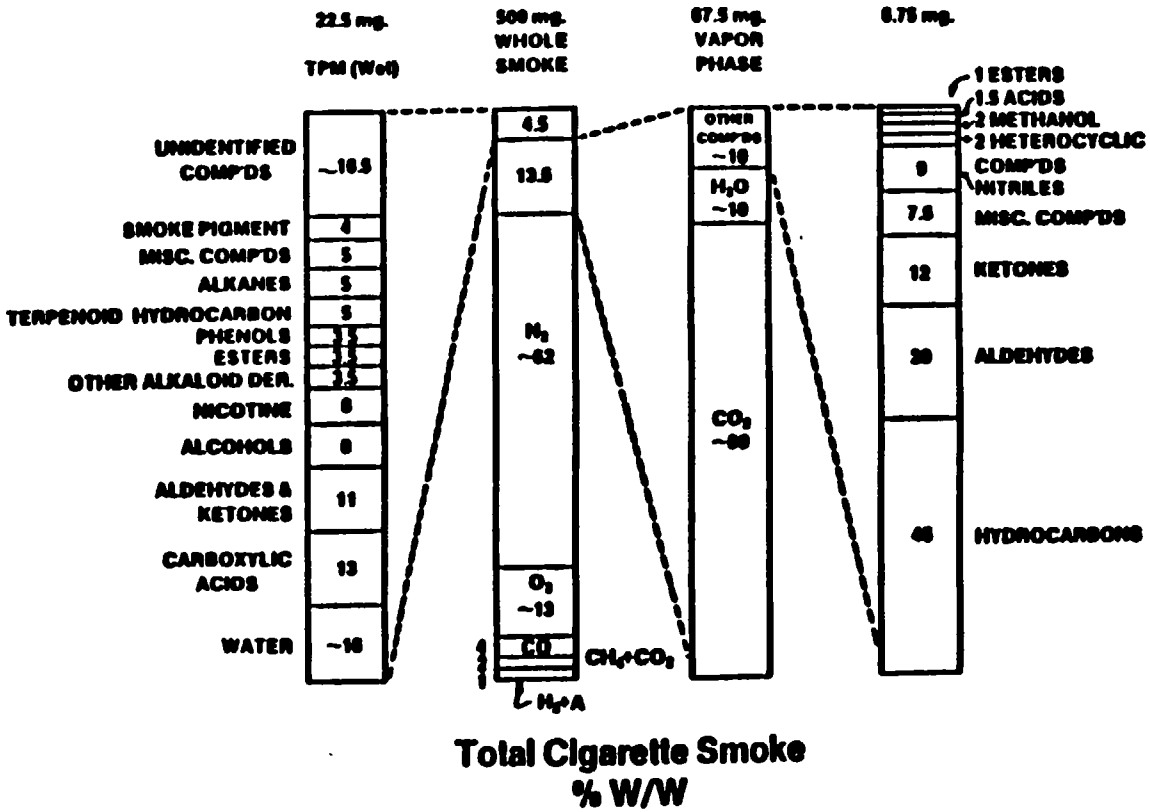
4. Diagram of Thermal Energy Analyzer (20)

5. Structures of Volatile N-Nitrosamines in Cigarette Smoke (20)

6. Carcinogenic Polynuclear Aromatic Hydrocarbons in Cigarette Smoke (16, 17)

7. Formation of Tobacco-Specific N-Nitrosamines (20)

Figure 1 Total Cigarette Smoke (% W/W; 2)



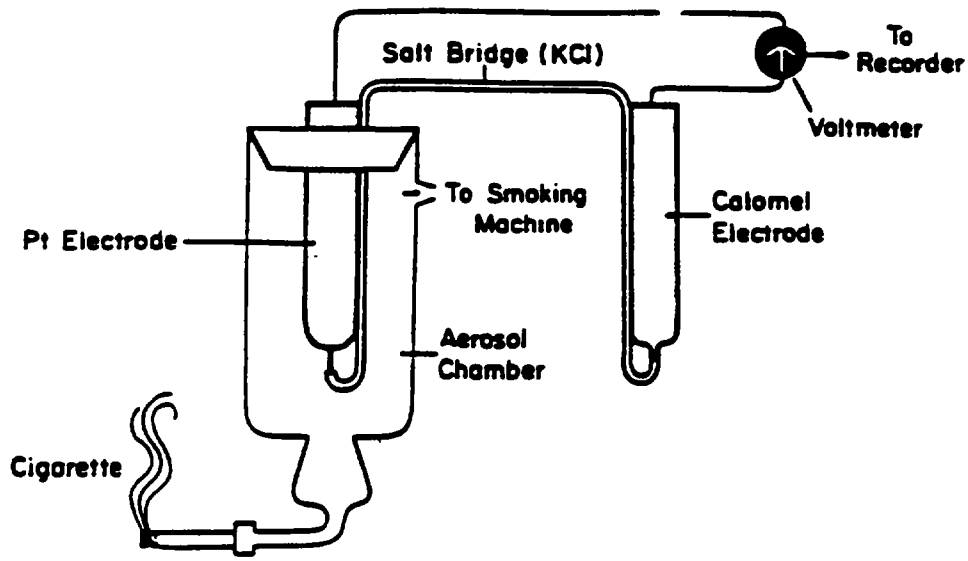


Figure 2 Apparatus for Measuring Redox Potential of Cigarette Smoke (30)

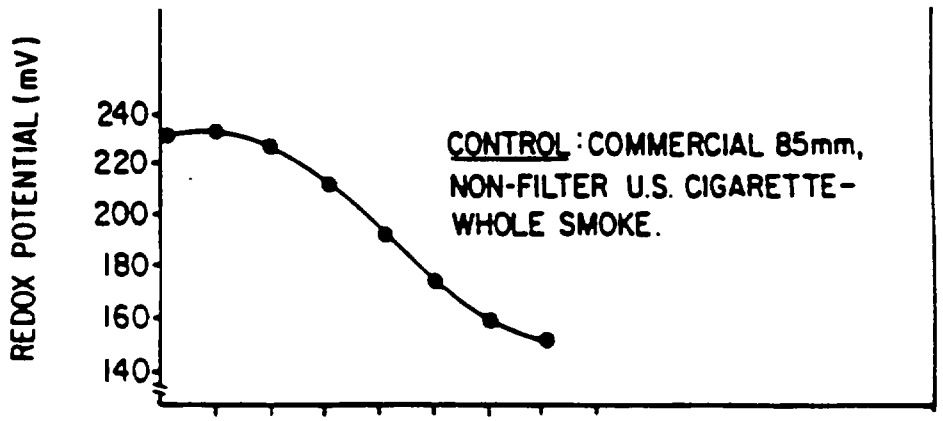
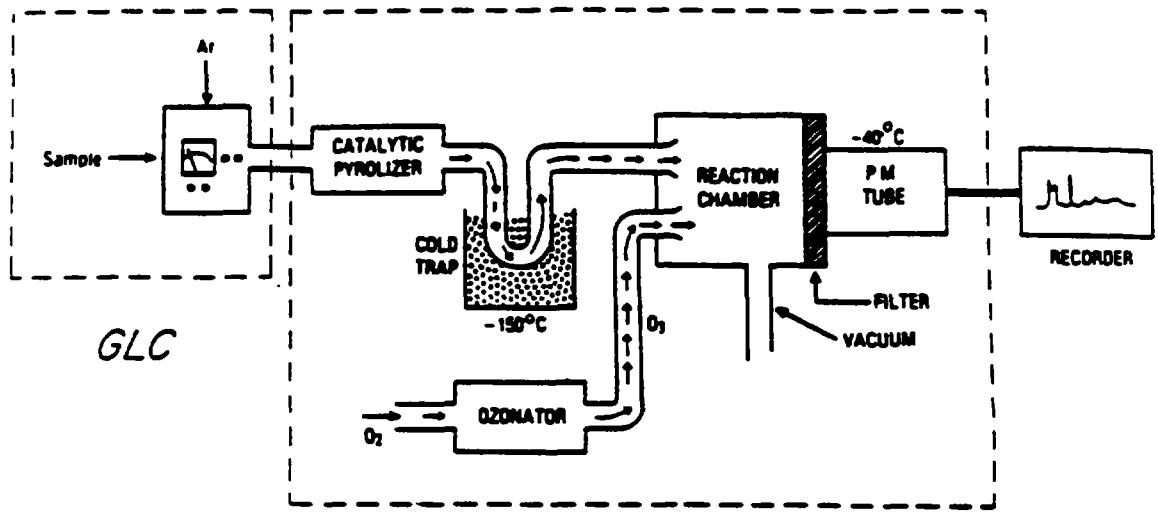


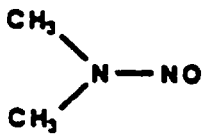
Figure 3 Redox Potential of Puffs 1 to 8 of an 85 mm Plain U.S. Cigarette (30)

Figure 4 Diagram of the Thermal Energy Analyzer (20)

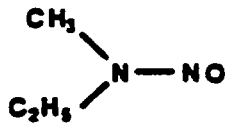


$$NO \xrightarrow{O_3} NO_2^* \longrightarrow NO_2 + h\nu$$

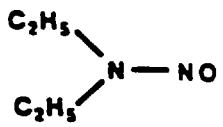
Figure 5 Volatile N-Nitrosamines (20)



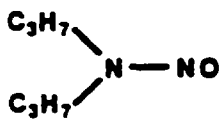
N-Nitrosodimethylamine (NDMA)



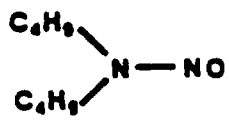
N-Nitrosoethylmethylamine (NEMA)



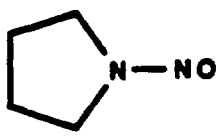
N-Nitrosodiethylamine (NDEA)



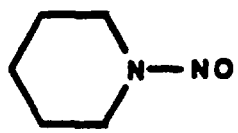
N-Nitrosodipropylamine (NDPA)



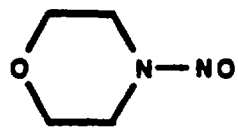
N-Nitrosodibutylamine (NDBA)



N-Nitrosopyrrolidine (NPYR)

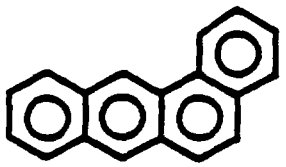


N-Nitrosopiperidine (NPIP)

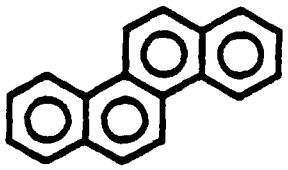


N-Nitrosomorpholine (NMOR)

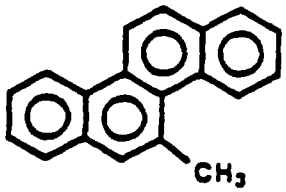
Figure 6 Carcinogenic Polynuclear Aromatic Hydrocarbons and Aza-Arenes in Cigarette Smoke (16, 17)



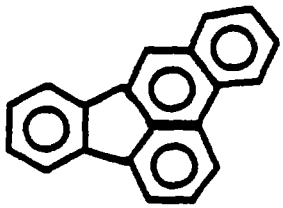
Benz(a)anthracenes



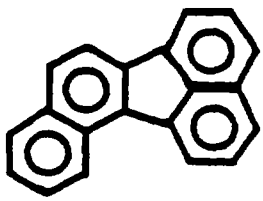
Chrysene



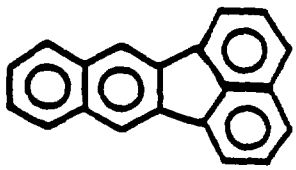
5-Methylchrysene



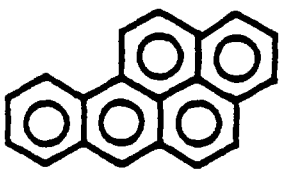
Benzo(b)fluoranthene



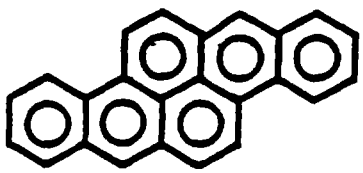
Benzo(j)fluoranthene



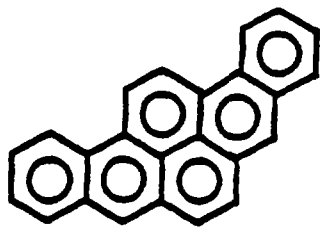
Benzo(k)fluoranthene



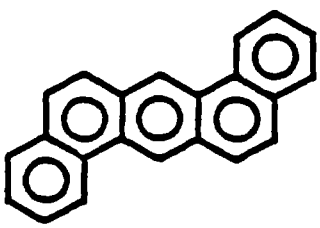
Benzo(a)pyrene



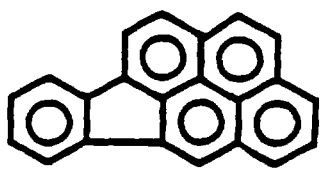
Dibenzo(a,h)pyrene



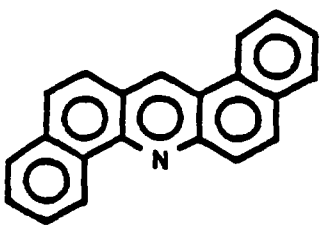
Dibenzo(a,i)pyrene



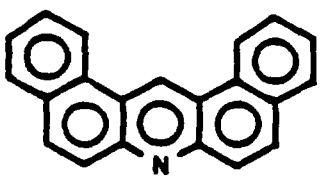
Dibenz(a,h)anthracene



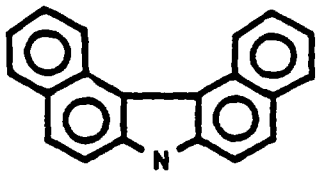
Indeno(1,2,3-cd)pyrene



Dibenz(a,h)aeridine



Dibenz(a,j)acridine



7H-Dibenzo(c,g)carbazole