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TOBACCO SMOKE ANALYSIS PROGRAM
PROGRESS REPORT FOR THE PERIOD
JANUARY 1, 1970, TO SEPTEMBER 1, 1970

Prepared by
M. R. Guerin
W. D. Shults

OAK RIDGE NATIONAL LABORATORY
operated by
UNION CARBIDE CORPORATION
for the
U.S. ATOMIC ENERGY COMMISSION

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ANALYTICAL CHEMISTRY DIVISION

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JANUARY 1971

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CONTENTS

SUMMARY.....	V
1.00 Introduction	1
2.00 Facilities and Special Equipment	3
2.01 Sample Storage	3
2.02 Controlled Environment Laboratory, Yellow Rooms.....	3
2.03 Smoking Machine One - Particulate Matter and Gas Phase...	4
2.04 Smoking Machine Two - Gas Phase	4
2.05 Smoking Machine Three - Gas Phase and Whole Smoke	5
2.06 Smoking Machine Four - Smoke Condensate	6
2.07 Filter Holder Leak Test Apparatus	7
2.08 Resistance-To-Draw Apparatus	7
2.09 Griffith Still	8
2.10 Electroanalytical Instrumentation	8
2.11 Element-Specific Gas Chromatography Detectors	9
2.12 Research and Routine Gas Chromatographs	9
2.13 Cryothermal Gas Chromatograph	10
2.14 Computational Facilities	10
2.15 Miscellaneous Equipment	11
2.16 Gas Chromatography - Tobacco Science Literature Collection	11
2.17 Chemical Bank	11
3.00 Analysis of Particulate Matter	12
3.01 Total Particulate Matter	13
3.02 Water in Particulate Matter	15
3.03 Alkaloids as Nicotine in Particulate Matter	17
3.04 Tar in Particulate Matter	18
3.05 Studies of the Data	18
4.00 Analysis of the Gas Phase	23
4.01 Acetaldehyde and Acrolein	24
4.02 Carbon Monoxide and Carbon Dioxide	27
4.03 Formaldehyde	27

4.04	Oxides of Nitrogen	28
4.05	Hydrogen Cyanide	30
4.06	Gas Phase pH	31
5.00	Analysis of the Condensate	33
5.01	Sampling Prepared Condensate	33
5.02	Water and Acetone	34
5.03	Nicotine and Nornicotine	35
5.04	Phenol and the Cresols	36
5.05	Phenanthrene, Benz(a)anthracene, and Benzo(a)pyrene	38
5.06	Weak and Very Weak Acids	40
5.07	pH of Condensate	41
6.00	Research Activities	42
6.01	N-Nitrosamines	42
6.02	Gas Chromatographic Component Profiles - Whole Smoke Condensate	43
6.03	Gas Chromatographic Component Profiles - Gas Phase of Smoke	44
6.04	Sulfur-Containing Compounds of Smokes	45
6.05	Phenols of Cigarette Smoke	46
6.06	Nitrogen-Containing Compounds of Smoke Condensate	47
6.07	Carboxylic Acids of Smoke	48
6.08	Polycyclic Aromatic Hydrocarbons of Smoke Condensate	48
6.09	Influence of Cigarette Storage Conditions on Chemical Delivery	51
7.00	References	54
8.00	Tables and Figures	56
9.00	Appendix A - Preliminary Procedures	112
10.00	Appendix B - Experimental Results	170
11.00	Appendix C - Operating Protocol	182

SUMMARY

Chemical assays are being established and applied to the smokes of a series of experimental cigarettes. The results of these assays, correlated with the results of bioassays carried out elsewhere, provide information to better define the relationship between cigarette characteristics and experimental tobacco carcinogenesis.

Cigarettes are smoked to determine total particulate matter, water, nicotine, and tar. The gas phases of the smokes are analyzed for carbon monoxide, carbon dioxide, acetaldehyde, acrolein, oxides of nitrogen, hydrogen cyanide, formaldehyde and acidity. Smoke condensates are analyzed for water, acetone, tar, nicotine, nornicotine, phenanthrene, benz(a)anthracene, benzo(a)pyrene, phenol, o-cresol, m-cresol, p-cresol, and acids. Other components may be determined on a non-routine basis.

Cigarettes have been smoked and the generated particulate matter has been analyzed to determine the tar and nicotine deliveries of all of the experimental cigarettes. The data has been studied in detail to provide measures of this facility's ability to generate precise, meaningful results and to allow ranking the experimental cigarettes according to their deliveries of particulate matter. Acetaldehyde and acrolein have been determined in the gas phases of the cigarettes. The formaldehyde and hydrogen cyanide contents of the gas phases are presently being determined. Other gas phase assays and some condensate assays will be applied to the experimental cigarettes shortly. Several assays require further verification before they can be confidently applied to the samples.

Research and development activities have heavily emphasized support of the routine operations. One result of these activities is an analytical system capable of excellent precision in the simultaneous determination of acetaldehyde and acrolein. Studies of the gas chromatographic properties of nicotine, nornicotine and monohydric phenols greatly facilitated the selection of chromatographic conditions for the routine analyses of these compounds. The application of a liquid chromatograph to the separation of phenanthrene, benz(a)anthracene and benzo(a)pyrene

suggests the possibility of greatly shortening the time required for the determination of these polycyclic hydrocarbons in condensates. A consideration of the characteristics of the cyanide-specific electrode suggests a superior approach to the determination of the hydrogen cyanide delivery of cigarettes. Experience with porous-polymer gas chromatography has facilitated the development of means for the determination of water in particulate matter, water and acetone in condensates, and has suggested the possibility of determining formaldehyde gas chromatographically. Activities with selective-detectors in gas chromatography have suggested a possible system for the determination of oxides of nitrogen in the gas phase of smokes.

Work in areas less directly related to the routine operations has also produced promising results. A study of the polarographic properties of N-nitrosamines suggests the possibility of the electrochemical determination of the N-nitrosamines in condensates. Trimethylsilylation of pyridine solutions of cigarette smoke condensate has been found to allow the reproducible generation of gas chromatograms displaying more than thirty of the major constituents of whole condensate in less than one hour. The direct application of a flame photometric detection system to the gas chromatography of the gas phase of cigarette smoke has allowed the visualization of hydrogen sulfide, carbonyl sulfide, carbon disulfide, thiophene and ten other sulfur-bearing smoke constituents in fractions of a puff of smoke.

The report summarizes the progress made in routine operations and research activities during the period of approximately Jan. 1, 1970 to Sept. 1, 1970.

1.00 INTRODUCTION

The Tobacco Smoke Analysis Program is designed to provide chemical support for the biological testing of a series of 23 experimental cigarettes. The availability of carefully defined chemical and biological data on the smokes of highly characterized cigarettes should allow confident assessments of the relationship between cigarette characteristics and experimental tobacco carcinogenesis.

The primary purpose of this activity at present is to provide the best possible values for the quantities of selected components of smoke particulate matter, gas phase and condensate. Frequent contact with members of the coordinating committee, the tobacco industry, private research organizations and public health organizations is necessary to accomplish this objective. Recommendations from all quarters are considered at this laboratory to determine their utility in providing superior quantitative analytical data. The final product of this activity will include highly defined analytical procedures which can be tested and applied elsewhere. Assessments of the precise biological significance of the quantities of components found present is left to those with proven experience in such endeavors.

Established procedures are applied whenever possible. The particular circumstances of this program, the requirement of a large number of analyses of widely differing kinds in a relatively short period of time, often influences the selection of procedures. Some established procedures must be modified to allow the generation of precise data in a reasonable period of time, yet satisfy the requirements of the coordinating committee. Other established procedures are rejected by this group when they are found unreliable in our hands. Although nominally "routine", few of the procedures in use in this effort enjoy the status of standard methods.

The extensive analytical chemistry facility at this laboratory often affords the opportunity to apply new methodologies to the determinations of components presently assayed by established but limited procedures. The facility also provides routes to the quantitative determination of important, but presently inaccessible components of smoke. New methodologies are examined and compared to those presently employed whenever possible.

This report summarizes the progress made in routine and research activities from the initiation of this effort on approximately January 1, 1970 to September 1, 1970.

2.00 FACILITIES AND SPECIAL EQUIPMENT

The facility in existence at the start of this program was not designed to meet the stringent cigarette handling and smoke generating requirements necessary for a successful routine operation. A considerable portion of the time and expenditures to date have been devoted directly toward establishing the necessary facilities. Additional acquisitions of hardware and equipment are expected to be minimal except for those cases where specific projects are supported or additional responsibilities of a routine nature are assigned.

2.01 SAMPLE STORAGE

Two freezers, polyethylene sheet and a heat-sealer are used for the long-term storage of samples. Cigarettes are removed from the shipping container within 36 hours of receipt, counted, heat-sealed in air-tight polyethylene bags in quantities according to expected usage and placed in a freezer at zero degrees fahrenheit. When needed, packets of cigarettes are allowed to come to room temperature while sealed and are opened in the controlled environment laboratory to begin conditioning.

Samples of condensate are transferred directly from the dry ice packing to the freezer without thawing.

2.02 CONTROLLED ENVIRONMENT LABORATORY, YELLOW ROOMS

A three-man limited-access laboratory has been equipped with temperature and humidity control systems. The relative humidity is maintained at $60 \pm 2\%$ and the temperature at $75 \pm 2^\circ$ fahrenheit. The temperature and humidity are monitored manually at least daily with a Bendix Psychron Wet Bulb Meter. A Taylor Recording Temperature-Humidity Meter provides a continuous reading of the approximate temperature and humidity. Adjustments are made as needed.

Standard fluorescent lights have been replaced by Sylvania No. F40GD yellow light tubes in the Controlled Environment Laboratory (CEL). The adjacent one-man laboratory and all areas used for the analysis of the polycyclic aromatic hydrocarbons are also equipped with yellow lights.

The CEL is used for all operations dealing with the conditioning, selecting, and smoking of cigarettes. As such, the CEL presently houses

four smoking machines, two single-pan balances, the resistance-to-draw apparatus, the leak-test apparatus and the cigarette conditioning facilities. A cryothermal gas chromatograph and a gas phase pH assembly are also used in the CEL.

2.03 SMOKING MACHINE NUMBER 1- PARTICULATE MATTER AND GAS PHASE

A four-port version (Fig. 2-1) of the Philip Morris 20-Channel Automated Smoking Machine is used for the generation of particulate matter and certain gas phase samples. Manufactured by Phipps and Bird, Inc., Richmond, Virginia, the four-port unit includes all those features characteristic of the larger machine. Puff volume inconsistency is decreased by a positive pressure syringe-based puff cycle. Inconsistencies in puff duration are lessened by use of an accurate electronic control of puff duration. Operator error is made less important since each smoking port is closed automatically when the cigarette char mark reaches the selected butt length on each cigarette. The puff number is displayed to approximately 0.1 puff by a counter. Fume hood draft variation is controlled by a vent equipped with an adjustable damper.

The same puffing mechanism and corresponding good control of puff volume, puff duration, and puff frequency is used for the generation of samples of certain gas phase components. Flow lines are attached from the syringes directly to the appropriate traps mounted on the back of the smoking machine. The filter assembly is mounted upstream of the trap such that the smoke passes through the Cambridge Filter before reaching the trap. The number of puffs required to reach a selected butt length must be estimated when the machine is used in this mode.

2.04 SMOKING MACHINE NUMBER 2 - GAS PHASE

A four-port smoking machine (Fig. 2-2, 2-3) was designed and constructed at this laboratory specifically for the high precision sampling of the gas phase of smokes for gas chromatographic analysis. The machine consists of four independent smoking mechanisms, any or all of which can be joined in parallel to provide samples of single puffs that are composites of from one to four cigarettes. Full puffs or any fraction of a puff can be sampled. The smoke of an entire cigarette can also be sampled provided a means exists to trap the smoke from each puff and thereby

accumulate the cigarette's total delivery.

A flow diagram of smoking machine number 2 is given in Fig. 2-3. Each smoke train consists of the cigarette, filter assembly, three-way solenoid operated valve, solenoid operated gas sampling valve, variable restrictor, and Dyna-Vac pump (The variable restrictor is erroneously shown upstream of the gas sampling valve in Fig. 2-3; it is actually located downstream of the valve). The smoke trains are tied together through the gas sampling valves with a helium line. Separate switches allow the operator to deactivate any of the gas sampling valves and thereby remove the corresponding smoke train from the sampling procedure.

In operation, an electric timer opens the three-way solenoid thus connecting the cigarette to the pump. Smoke is drawn through the smoke train and fills the sample loop. Precisely two seconds after the puff begins, the three-way solenoid closes and stops the puff. As the puff stops, the gas sampling valve switches to the "transfer" mode and helium sweeps the contents of loops into traps or onto a gas chromatographic column. Three seconds before the next puff, the gas sampling valve switches to the "sample" mode to release the helium pressure through the three-way valve without affecting the cigarette. The cycle then repeats until the operator removes the cigarette butt from the filter assembly.

The only complication noted to date is that the orifices of the gas sampling valve very nearly limit the flow of air to that required in smoking (35 ml in two seconds). As a result, the variable restrictor provided poor control in adjustments of the puff volume. This complication was eliminated when the orifices of the sample valve were made larger. Judging from the puff numbers found for given cigarettes with the Phipps and Bird machine and those reported by R. J. Reynolds for the experimental cigarettes, this smoking machine operates properly. Frequent checks of the puff volume and duration indicate there is no difficulty in maintaining the appropriate smoking parameters.

2.05 SMOKING MACHINE NUMBER 3 - GAS PHASE AND WHOLE SMOKE

A third smoking machine was fabricated specifically for use in generating samples for the determination of carbon monoxide and carbon dioxide in the gas phase of smokes. Its characteristics made the machine

useful for the generation of other gas phase samples as well. At present, it also serves as the best smoking mechanism available to this group for the sampling of whole smoke.

A photograph of the smoking machine is presented in Fig. 2-4. The puff mechanism is a positive puff syringe-based system comparable to that employed in the standard Phipps and Bird machine. A 130 cc capacity Model AB-5 Filamatic Vial Filler (National Instruments Co., Baltimore, Md.) equipped with a stainless steel syringe and micrometer volume control was modified to ensure a precisely 360° rotation in four seconds with a 60-second interval between rotations (puffs). In operation, the cigarette is lit at the beginning of the first syringe rotation and smoking continues automatically until the operator removes the cigarette from its holder. The puff number must be estimated by the operator.

Frequent checks of the puff volume, puff duration and puff frequency indicate that the appropriate smoking parameters are reproducible and easily controlled. The number of puffs required to reach the 23 mm butt length for the 23 experimental cigarettes have been essentially the same as with the other machines in use by this group.

2.06 SMOKING MACHINE NUMBER 4- SMOKE CONDENSATE

This group recently received the Model IIIa Automatic Smoking Machine pictured in Fig. 2-5 from Heinr Borgwaldt of Hamburg, Germany. This machine will find extensive use in the production of smoke condensates from the 23 experimental cigarettes.

The machine automatically smokes 30 cigarettes at a time under standard conditions of puff frequency, puff duration, and puff volume. Counting, feeding, and lighting of the cigarettes as well as ejection and extinction of the butts are all done automatically. As many as 1250 cigarettes can be smoked in an 8-hour day.

The single deviation from standard practice is that cigarettes are smoked with a constant number of puffs rather than to a given butt length. This deviation from standard practice is permitted when 500 or more cigarettes are smoked.

The single large-volume cold trap is designed for the condensation of the smoke of up to 6000 cigarettes. No filter materials or solvents are used.

2.07 FILTER HOLDER LEAK TEST APPARATUS

An apparatus assembled and in use here for rapidly testing Cambridge Filter Assemblies for major leaks is diagrammed in Fig. 2-6. In operation, a standing column of water is produced by opening the vacuum line. A Cambridge Filter Assembly, plugged where the cigarette is normally inserted, is attached to the test side of the apparatus and the line is opened to the filter assembly. A leak is evidenced by a rapid drop of the standing water column.

Applications of this apparatus have shown that leaks rarely occur when rubber o-rings are used in the filter assemblies. Filter assemblies using Teflon gaskets leak frequently. Rubber o-rings are used exclusively in our work.

2.08 RESISTANCE-TO-DRAW APPARATUS

An instrument, identical in principle to that described by Wynder and Hoffmann (33), was fabricated to measure resistance-to-draw. This device differs from most others in that resistance-to-draw is measured by a Magnehelic gauge with a range of 0 to 12 inches of water pressure. Flow through the system is controlled by a "Nupro" needle valve and a rotameter is used to measure the flow rate. The rotameter was calibrated by a George K. Fisher Vol-U-Meter. A flow diagram of the instrument is shown in Fig. 2-7, and a photograph of the face of the instrument is shown in Fig. 2-8. Air is drawn through the system by a Dyna-Vac pump, and the rotameter float is set by means of the Nupro valve to give a flow through the system of 1050 cc per min. It was found that the rotameter float rides the pressure peak created by the pulsing diaphragm of the Dyna-Vac pump. The flow indicated by the float is much higher than the actual flow. This problem was corrected by inserting a surge tank into the pressure side of the system between the pump and the rotameter. The dial of the Magnehelic gauge is set at zero, and the cigarette is inserted in the "lip" of the cigarette holder. Resistance-to-draw may be read precisely to the nearest 0.1-in, and estimated to the nearest 0.01-in.

Data for the experimental cigarettes are given in Table 2-1.

2.09 GRIFFITH STILL

A rapid, reliable separation of the total alkaloids from tobacco, total particulate matter, and smoke condensate for subsequent quantitative ultraviolet spectrophotometric determination as nicotine is possible with the use of a Griffith Still (Fig. 2-9). A preliminary steam distillation from a slightly acidic medium separates the volatile phenol fraction (21) which also can be analyzed quantitatively. A subsequent steam distillation from strong alkali enriches the total alkaloids.

The make-up of the Griffith steam distillation apparatus has been described in great detail (9). A unit consists of a Pyrex still pot of approximately 50 ml capacity, a glass connecting tube, a 2-bulb offset trap system, a brass condenser, and an overflow U-tube receiver. Heat is supplied by Glas-Col heaters encasing the still pots. Steam is introduced from a manifold into the still pots through a 3-way Teflon capillary stopcock located on the bottom of the pot.

The complete Griffith Still consists of six such basic units housed in an open-faced cabinet. The cabinet provides convenient outlets, inlets, and controls for electrical power, steam, and waste water removal.

The Griffith Still has performed excellently in well over 200 distillations carried out thus far.

2.10 ELECTROANALYTICAL INSTRUMENTATION

An array of electroanalytical equipment is in use in both routine and research activities. A Beckman Expandomatic pH meter equipped with a Beckman No. 39183 combination electrode is employed for determining the pH of smoke condensates. A modified Beckman No. 39183 combination electrode coupled to a Heath pH recording electrometer constitutes the sensing-displaying portion of the gas phase pH assembly. A Model E336 Metrohm Herisau Potentiograph is in use for the potentiometric titration of weak and very weak acids in condensates.

Orion nitrate and cyanide ion-selective electrodes have been used with Beckman Expandomatic and Beckman Research pH meters in studies of alternative methods for the determination of oxides of nitrogen as nitrate and hydrogen cyanide as cyanide. Other ion-selective electrodes are also available if needed.

The full spectrum of electroanalytical techniques are accessible through Methodology and Analytical Instrumentation Groups of this Division. Instrumentation and expertise in all forms of polarography, coulometry, amperometry, and potentiometry are available as the need arises. Polarographic methodologies have already been utilized in studies of hydrogen cyanide and N-nitrosamines.

2.11 ELEMENT-SPECIFIC GAS CHROMATOGRAPHY DETECTORS

The research gas chromatograph available for this effort is equipped with a Ni-63 electron capture detector, Melpar flame photometric detector, and Coulson electrolytic conductivity detector in addition to the common hydrogen flame ionization detector. The electron capture detector is particularly useful for the detection of trace quantities of polycyclic aromatic hydrocarbons, but may also be used for the analyses of trace quantities of any smoke constituents which are electron capturing species or can be made capturing species by suitable derivatization techniques. The photometric detector is particularly useful for the detection of phosphorus-and-sulfur-containing components (see Section 6). The conductivity detector, operated in the nitrogen specific mode, shows great promise for the analyses of components such as nitrogen-heterocyclics, N-nitrosamines, oxides of nitrogen, and hydrogen cyanide.

2.12 RESEARCH AND ROUTINE GAS CHROMATOGRAPHS

A MicroTek Model 160 dual-column gas chromatograph is available as a research instrument. In addition to housing the selective detectors mentioned in Section 2.11, features include the ability to work with an all glass flow train, to operate four columns at a time, and to operate with linear temperature programming. Flash vaporization and on-column injection are both possible. Because of the large number of determinations which must be carried out gas chromatographically, the Model 220 sees considerable service as a routine analytical instrument.

A MicroTek Model 160 dual-column gas chromatograph is in constant use as the primary routine instrument. The Model 160 is equipped with a hydrogen flame ionization detector, thermal conductivity detector, and linear temperature programmer. Considerable savings was realized by modifying the carrier gas flow lines, and designing the column characteristics so that two analyses could be carried out simultaneously. At present

water in particulate matter or water and acetone in condensate preparations can be determined using one column while the second column is used for the determination of nicotine. A slight adjustment of conditions allows the same system to be used for the analysis of phenols.

2.13 Cryothermal Gas Chromatograph

A MicroTek Model 150 gas chromatograph is shown coupled to the four-port gas phase smoking machine (Section 2.04) in Fig. 2-2. The Model 150 is equipped with a CT-75 Cryothermal system which allows the column to be operated at sub-ambient temperatures. The ability to operate isothermally or program the column temperature at any point from liquid nitrogen temperature to 400°C allows the use of the column as an efficient trap and makes this system particularly suitable for gas phase analysis.

The cryothermal gas chromatograph is presently equipped with dual, six-port gas sampling column switching valves, a hydrogen flame ionization detector and a thermal conductivity detector. Housed in the controlled environment laboratory, the cryothermal gas chromatograph is in use for the analysis of acetaldehyde, acrolein, carbon monoxide, and carbon dioxide in the gas phase of smokes. This system, particularly when coupled to the four-port smoking machine, offers many opportunities for research and development on the composition of fresh smoke.

2.14 COMPUTATIONAL FACILITIES

The results of the various determinations are computed as soon as possible after each analysis with the aid of two Marchant mechanical calculators. A Hewlett-Packard electronic programmable calculator available to this group has been employed to compute standard deviations when large numbers of values must be considered. The average weights and corresponding standard deviations of fifty cigarettes of each code (Table 2-2) were computed using the programmable calculator. A substantial amount of time has been required to calculate the results and precision of each analysis and to express these results in different units. It may be advantageous to make use of this Laboratory's extensive computer facility to lessen the times involved and minimize the opportunities for errors to occur.

2.15 MISCELLANEOUS EQUIPMENT

Arrangements have been made to have access to that equipment necessary to carry out column, paper, and thin-layer chromatography. Glassware and supporting equipment for extractions and concentration procedures are also on hand. A Cary 14 spectrophotometer and a Beckman B spectrophotometer are in use in those methods relying on absorption spectrophotometry. A Perkin-Elmer Model 203 Fluorescence Spectrophotometer is in use for the analysis of the polycyclic aromatic hydrocarbons. The Analytical Chemistry Division's extensive liquid scintillation counting facility is available for counting carbon-14 tracers.

2.16 GAS CHROMATOGRAPHY-TOBACCO SCIENCE LITERATURE COLLECTION

Exhaustive computer searches of Gas Chromatography Abstracts and of Chemical Titles culminated in a collection of references, abstracts and/or publications dealing with the application of gas chromatography in the tobacco sciences. A thorough study of the publications comprising the collection has been initiated. The chromatographic conditions and results described in pertinent papers are being tabulated. Tables entitled "Chromatographic Conditions", "Retention Characteristics", "Sample Characteristics", and "Quantity in Smoke" presently contain entries from a study of approximately 150 of the 300 publications in our file. The tables provide a convenient survey of the gas chromatographic methodology thus far utilized in tobacco science, and function as a thorough index to our literature.

The tables are not yet in final form. Additional updating of the file is necessary to insure a representative sampling of the past year's publications.

2.17 CHEMICAL BANK

A collection of chemicals of importance in tobacco science has been established to facilitate experimental efforts. Of the chemicals selected as the core of this collection, approximately 200 are now on hand. Included in those now available are phenols, acids, amines, polynuclear aromatic hydrocarbons, nitrogen heterocyclics, N-nitrosamines, inorganic gases and low molecular weight hydrocarbons.

3.00 ANALYSIS OF PARTICULATE MATTER

The particulate matter generated by a burning cigarette corresponds to that portion of the smoke that can be collected by a Cambridge CML13A glass-fibre filter. Physically, this corresponds to 99.9% of the generated particles 0.3 micron and larger in size. The gross increase in weight of the filter attending the smoking process is termed the "total particulate matter". The filter pad is analyzed to determine its water content and nicotine content. The "tar" delivered by the cigarette is then determined as the difference between the weight of total particulate matter and combined weights of water plus nicotine.

The determinations of tar and nicotine deliveries of the cigarettes rank among the most important analyses of this program. Tar and nicotine levels are the only parameters that the majority of health scientists regard as definitely correlateable with the health hazards presumably associated with cigarette smoking. None of the other analytical procedures have been studied as extensively to determine their reliability. None of the other parameters determined offer the promise of allowing the experimental cigarettes to be "ranked" chemically in order of anticipated general toxicity. With tar-nicotine data now being regularly generated for commercial cigarettes, the results of these analyses of the experimental cigarettes provides a means of roughly comparing the commercial and experimental cigarettes.

Because of its importance and the fact that it is highly characterized, this analytical procedure has been studied in substantial detail here. The availability of comparable data for the experimental cigarettes from a collaborating laboratory allows a measure of the general inter-laboratory agreement anticipated for these samples. The availability of published studies of the within-laboratory and between-laboratory precision of tar-nicotine determinations allows a measure of the capability of this facility to reproducibly smoke cigarettes and to precisely generate such data.

General discussions of the various aspects of the procedure are presented below. Detailed procedures are appended to this report. This section concludes with a summary of the important conclusions that result from a study of the generated data.

3.01 TOTAL PARTICULATE MATTER

The determination of total particulate matter (TPM) involves determining the increase in weight of the Cambridge filter upon smoking a cigarette (generally, four cigarettes per filter). This "analysis" is as much a sample preparation as it is an analysis. An error in this phase of the tar-nicotine determination is carried throughout the analytical procedure. An error in or poor control of any of the variables associated with cigarette storage, conditioning, selection and smoking can produce an error in the value for TPM and an unrepresentative sample of particulate matter.

Insufficient work has been done to assess the influence of variations in cigarette storage procedures on tar-nicotine deliveries (see Section 6.09). It would seem reasonable that simply freezing unsealed cigarettes would afford the possibility of a freeze-drying phenomena which might result in a substantial change in the cigarettes. Our practice has been to first heat-seal quantities of cigarettes in polyethylene sheet before freezing. The cigarette packets are allowed to come to room temperature before they are opened in the hope that the water or other components that may have entered the air space would re-distribute throughout the cigarettes before conditioning begins. The cigarettes are opened to the conditioning room after at least several hours at room temperature.

The cigarettes are placed loosely in a wire-mesh tray and exposed to the conditioning environment of 60% RH and 75°F. The length of time necessary to insure that the cigarettes are conditioned is largely a function of the rate of flow of conditioned air around the cigarettes. The rate at which conditioned air moves across the cigarettes is, in turn, a function of air turn-over rate in the conditioning room and the location of the cigarette trays. We have found that a 24-hour conditioning time is barely adequate in our conditioning room. As a general practice, we place the trays on a table where normal convection is unobstructed and weight select the cigarettes to ± 20 mg of their average weight after 24 to 36 hours. The selected cigarettes are stored in the conditioning room for an additional 12 to 24 hours before smoking. We note no appreciable change in weight of the cigarettes after approximately 30 hours.

The smoking process is, of course, critical. Puff volume, duration and frequency can be carefully controlled using our Phipps and Bird four-port machine. Puff duration is set at two seconds by adjusting the unit so the syringe completes a full cycle in four seconds. Puff frequency is held at one per minute by the timing mechanism. Puff volume is set at 35 ml through the filter pad by adjusting the syringe stroke. Puff volume is checked daily by attaching a bubble meter to the filter assembly and observing the displacement of the soap film upon manual activation of the syringe.

Cigarettes are ignited with electric lighters supplied with the machine. Midway through the analyses reported on 8-24-70 the electric coils malfunctioned, forcing the use of matches for ignition. Several repeated analyses of samples previously ignited by electric lighter indicated no detectable difference in tar-nicotine deliveries resulting from the change in lighting procedure.

The preferred fume hood velocity was not specified by the program coordinators. The full series of experimental cigarettes were analyzed using the smoking machine manufacturer's recommended velocity of approximately 300 cubic feet per minute. A comparison of the number of puffs required to reach a 23 mm butt at ORNL with those published for the Kentucky Reference Cigarette and those provided by the collaborator, suggested that 300 cfm was excessive for the four-port machine in our laboratory environment. The complete series was analyzed again using a fume hood velocity of approximately 60 cfm. The Kentucky Reference Cigarette was smoked daily as a monitor cigarette during the low velocity generation. Only one minor adjustment was required to provide the appropriate burn rate (11.1 ± 0.2 puffs for a IRI cigarette weighing 1.100 grams) during the three weeks of analyses. Agreement of puff numbers with those reported by the collaborator was better at low hood velocity (see Section 3.05).

A detailed procedure for determining the TPM delivered by cigarettes is given in Section 9.01. We report a value for dry TPM of 39.4 mg/cig. for the Kentucky Reference Cigarette as compared to a reported (25) mean value of 38.5 (range from 33.6 - 41.6). We find an average relative

standard deviation of 2.3% (see Section 10) for the determination of TPM over the series of experimental cigarettes which compares favorably with the precision reported (22, 24, 25) for several within-laboratory studies (see Table 3-2). Agreement with the collaborator is within an average of 3.1 mg/cig for the TPM of the experimental cigarettes which is also in accord with published studies (see Section 10).

3.02 WATER IN PARTICULATE MATTER

Water is determined in particulate matter to allow expressing TPM and tar results on a dry basis. This is expected to increase inter-laboratory agreement by correcting for slight differences in the water contents of the cigarettes from laboratory to laboratory. It is also important to know how much of the weight attributed to "tar" is actually "tar" and neither nicotine nor water.

The filter pads are removed from the filter assemblies immediately after TPM is determined, placed in vials, and capped with septums. Twenty milliliters of dry p-dioxane containing 0.3% isopropanol are carefully added to each filter-containing vial. A blank is prepared by contacting one and one quarter unsmoked filter pads with the same volume of solvent. Aliquots of the extracts are gas chromatographed according to Schultz and Spears (29) after standing in contact with the filters overnight.

Gas chromatographic columns of Porapak Q, Porapak S and Chromosorb 101 were examined for their utility in this determination. It was of interest to learn whether a given column material might prove useful for both the determination of water in particulate matter and water-acetone in condensate using isopropanol as the internal standard. Of the columns tested, only Porapak S resolved isopropanol from acetone (see Fig. 3-1). The time required for the separation, and the fact that resolution was not complete suggest that a better approach is to use a different internal standard for the determination of water and acetone in condensate. All of the columns examined provide adequate resolution for the use of absolute ethanol as an internal standard in the water-acetone determination.

The column system selected for the determination of water in particulate matter is a three-foot length of 0.25-in o.d., standard wall, copper

tubing packed with 80-100 mesh Porapak Q. Examples of chromatograms generated by standard solutions are given in Fig. 3-2. Sample extracts produce comparable chromatograms. The precise column temperature to be used depends upon the particular batch of Porapak Q used. Temperatures from 170-190°C have been employed with supposedly identical columns in order to generate chromatograms such as that given in Fig. 3-2. We have noticed no difference in performance between copper and stainless steel columns.

The stability of Porapak Q column has been good. At least four hundred injections of 10 μ l dioxane aliquots have been made without noticeable affect on the separation or the quantitative reproducibility of the peak heights. Poor quantitative reproducibility of the peak heights has been the first indication of a deteriorated column. Our experience with Chromosorb 101 columns is that they have a substantially shorter lifetime in this application.

Approximately 30 calibration curves have been generated thus far. In the absence of errors, relative peak heights are sufficiently reproducible from day to day to make the calibration curves indistinguishable. We have often obtained virtually identical calibration curves for different preparations of the dioxane-isopropanol extractant. The ease with which the extractant, vials, and septum caps can pick up water frequently results in one poor calibration point and occasionally in a poor calibration curve.

The gas chromatographic portion of this analytical procedure appears to be well established. Details of the procedures involved in preparing the standards, preparing samples, and handling extracts require more attention. At present, the overall procedure appears capable of producing results with an average relative standard deviation of approximately 6%.

A detailed procedure for the determination of water in particulate matter is given in Section 9.03. Applications of the method thus far have produced results which agree with those of the collaborator within an average of 0.6 mg/cig.

3.03 ALKALOIDS AS NICOTINE IN PARTICULATE MATTER

A procedure comparable to those described by Pillsbury (24), and by Ogg (22) has been employed for the semi-routine separation and subsequent spectrophotometric determination of alkaloids as nicotine. A Cambridge filter pad that contains the total particulate matter from four cigarettes is contacted for 16 or more hours with 20 ml of 99.7% dioxane - 0.3% iso - propanol solution. After the percentage of water is determined, a 10 ml aliquot of the extract is steam distilled from strong alkali into an acidic medium using the Griffith Still. A portion of the distillate is then analyzed spectrophotometrically with a Cary Model 14 spectrophotometer. The total concentration of alkaloids in the distillate is calculated (as nicotine) from the absorbance readings.

The use of an aliquot of the extractant rather than distilling directly from the filter pad is the single departure from the methods of Ogg and of Pillsbury. Quantitative transfers of the whole filter pads produced volumes of solvent in the still pots large enough to result in blow-by. The stills were frequently plugged by the filter pad being thrown into the throat of the still. Results obtained by sampling the extractant were found to be the same as those obtained from distillations of the whole filter pad. In addition to being more convenient, the aliquot method allows the chemist to carry out duplicate analyses on a single port if necessary. A detailed procedure for determining the nicotine delivered by cigarettes is given in Section 9.02.

We report a value for nicotine of 2.74 mg/cig for the Kentucky Reference Cigarette as compared to the reported (25) mean value of 2.63 mg/cig (range, 2.16 - 2.98). We find an average relative standard deviation of 2.99% (see Section 10.00) for the determination of nicotine over the series of experimental cigarettes. This compares favorably with the precision reported (22, 24, 25) in several published studies (see Table 3-2). Agreement with the collaborator is within an average of 0.15 mg/cig for the nicotine delivery of the experimental cigarettes which is also in accord with published studies (Table 3-3).

3.04 TAR IN PARTICULATE MATTER

The tar content of particulate matter is determined by subtracting the weights of nicotine and water from the weight of total particulate matter. The values for tar generated at ORNL for the experimental cigarettes differ from those found by the collaborator by an average of 2.7 mg/cig. This compares well with the range of 7.4 mg/cig reported (25) for the inter-laboratory study of the Kentucky Reference Cigarette. The average relative standard deviation found for the determination of the tar deliveries of the experimental cigarettes was 2.4%.

3.05 STUDIES OF THE DATA

The tobacco smoke analysis facility established at Oak Ridge National Laboratory was new and largely untested. It is essential that the true capability of this facility to produce meaningful, precise, and reproducible values for the chemical species of interest be ascertained as early as possible. The successful generation of the required data demands that samples of smoke be generated with the utmost care and that an undefinable "feel", attainable only with experience, for the smoking process be a part of the facility's capability. The generation of samples of particulate matter is the most standardized, and thus provides the best testing ground of this facility's capability to accomplish its objective of generating meaningful quantitative measures of tobacco smoke constituents. As a result and because of the general importance of the tar-nicotine determinations, the data have been very thoroughly studied. Tables summarizing these studies are included in this report.

Largely through the kind cooperation of personnel involved in research and routine operations at Phillip Morris, Inc., and R. J. Reynolds Tobacco Products Division as well as Dr. R. B. Griffith of the Tobacco and Health Research Institute, Lexington, Kentucky, the primary conclusion of these studies is that this facility is capable of producing data comparable to that produced in the established tobacco laboratories. Comments and summary conclusions of the study of this data follow.

a. A study was made of the precision with which the data could be generated. The precision we operate within for a given series (4 ports, 4 cigarettes per port smoked simultaneously, and from day to day) (the same code handled in the same way, but smoked at different times over a three-week period) is illustrated in Table 3-1. The day to day results include subtle experimental differences expected to occur in actual practice — slightly different average cigarette weight, slightly different fume hood velocity, and slightly different conditioning times. The result of this study is that the standard deviation from day to day is approximately twice that for a given series. Except for the determination of water, the relative standard deviation is always less than 5%.

The standard deviations reported with each result define how precisely a given value for TPM, nicotine, etc., was generated in a given laboratory at a given time. All sixteen cigarettes are smoked over a time interval of 60 minutes or less. It would appear that only if all 23 samples could be smoked in a given 60-minute period on the same machine, then the tabulated standard deviations could set the true extremes of uncertainty for each value.

b. The precision with which results are generated at ORNL corresponds to that published in the literature. The results of this study, summarized in Table 3-2 suggest that we are capable of a precision comparable to that attainable elsewhere.

The "within-laboratory" data presented for ORNL corresponds to that for a given series of samples. It is implied in the publications of Ogg and of Pillsbury that the data used to obtain standard deviations in their studies were also from a given series, i.e., generated at a given time. The "between-laboratory" data presented at ORNL corresponds to that for day to day variability. One would anticipate better precision than for the true inter-laboratory case where different personnel are responsible for the operations. At worst, the data presented for ORNL "between-laboratory" would be compared to the "within-laboratory" results of others. Acceptable levels of precision are indicated in either case.

c. An attempt was made to learn whether the agreement between ORNL and the collaborator was reasonable. With only two values to compare there is no alternative, but to simply examine the difference for each sample code. A general measure of the agreement can be obtained by comparing this difference with the ranges of the results reported in published inter-laboratory studies, Table 3-3. It can at least be said that we agree as well or better than the commonly obtained inter-laboratory agreement. Seventeen of the 23 samples were smoked and analyzed more than once. The average range found for these multiple determinations (Tables 3-4 to 3-11) was: puff number = 0.28, TPM = 1.1, water = 0.5, nicotine = 0.11 and tar = 0.7. This would suggest that we disagree with the collaborator more than can be accounted for by a lack of reproducibility.

d. We find that if a given sample code is smoked and analyzed at various times over a period of three weeks and the results are pooled, averaged, and the standard deviation is computed, the standard deviation for the results is approximately twice the deviation for a given day's run and somewhat better than that reported for the best inter-laboratory agreement. It would appear more reasonable in attempts to rank the cigarettes in terms of nicotine and tar deliveries to consider the uncertainty of the results to be at least twice that suggested by the tabulated standard deviations.

The experimental cigarettes are ranked according to their average deliveries of TPM, water, nicotine and tar in Tables 3-12 and 3-13. Tables 3-14 and 3-15 present rankings of the cigarettes according to their deliveries of nicotine and tar considering not only the average levels found, but also uncertainties of $\pm \sigma$ and $\pm 2\sigma$. It is obvious that the relative deliveries of the cigarettes depend both on the units used to express the data and on the certainty one supposes for the results.

Table 3-16 presents the ranking of the cigarettes on the basis of the average deliveries of nicotine expressed on a milligrams per cigarette basis as determined at ORNL and by the collaborator. One sees that the agreement between the laboratories is quite good when an uncertainty of $\pm \sigma$ is supposed. Only the relative deliveries of cigarettes

number 4 and 8 are in substantial disagreement.

Table 3-17 presents the results of the TPM, water, nicotine and tar analyses expressed as milligrams per puff of the respective component. Table 3-18 ranks the cigarettes according to average deliveries on a milligrams per puff basis. The cigarettes are seen to be much less different (carefully refer to Table 3-17 when considering the data in Table 3-18) from one another when considered on this basis. The order of delivery is substantially different than found when the delivery per cigarette is considered.

e. A study of the data indicates that, generally, a fewer number of puffs are required to reach a 23 mm butt here than in the collaborator's laboratory. Yet, deliveries of tar and nicotine determined here are higher in general than found by the collaborator. This would appear to be a contradiction. A study of the results reported in the literature for inter-laboratory comparisons reveals that it is common to find laboratories reporting lower puff numbers yet higher deliveries than other laboratories.

f. Substantial differences in the number of puffs required to smoke a cigarette as reported by the two laboratories would cast doubt on the comparability of the results. An examination of the data reveals that a difference greater than one puff is reported for five of the 23 cigarettes. Each of these five codes were smoked more than once at this laboratory. Although some of these runs were neglected in computing the reported result because of other factors, the average puff numbers for each set of 16 cigarettes were reasonably reproducible. These values were: code 7-- 10.1, 9.6, 9.8; code -- 10.9, 11.0; code 15 -- 7.8, 7.0, 7.6, code 18-- 6.9, 6.8, 7.2; code 23 -- 8.8, 8.8, 9.0. The results found for the gas phase analyses compare favorably with those given above.

g. Tables 3-19 and 3-20 detail the results obtained for the Kentucky Reference Cigarette and experimental cigarette number 2 during a consideration of the influence of slight differences in experimental conditions on the results of the analysis of particulate matter. It was found that the statistical Q-test did not allow neglecting any of the individual values. Interestingly, the gross averages given in Tables

3-19 and 3-20 for puff number, TPM, water, nicotine, and tar are in reasonable agreement with the values reported (Section 10.00) using carefully studied data.

h. The average agreement found between ORNL and the collaborator for the ORNL data generated at high fume hood velocity and that at lower velocity is summarized in Table 3-21. It is seen that agreement is better for puff number and water, the same for nicotine and poorer for TPM and tar when the low velocity data are considered than the high velocity data.

The data appears to be consistent. An increased number of puffs resulting from the lower fume hood velocity would be expected to increase the TPM and tar delivery relative to the high velocity data. As the results reported by ORNL under high velocity conditions were slightly higher than the collaborator's results, an increased delivery would be expected to increase the difference between the laboratories. An increase of a fraction of a puff would not be expected to produce as large an increase in the total delivery of nicotine since the delivery of nicotine per puff is much smaller than for TPM or tar. A comparable argument would at least partially explain the water data.

4.00 ANALYSIS OF THE GAS PHASE

The gas phase of cigarette smoke is that portion of the smoke which passes through the Cambridge glass fiber filter. This phase includes those components which are gases at room temperature and those comprising the particles smaller than 0.3 micron. Components such as formaldehyde and acetaldehyde that are liquids at room temperature but possess high vapor pressures which are present both in the vapor state and as particulate matter. Such liquids are primarily found in the "gas phase" of smokes. The chemical complexity of the gas phase of smokes has been amply demonstrated by Grob (12).

No trap configuration or trapping system presently exists which will completely retain the entire gas phase of smokes in such a way as to produce a sample which can be aliquoted for the determination of all of the gas phase components of interest in this program. Trapping systems must be designed for the specific collection of the individual component of interest. An added complication results from the fact that the gas components can be irreversibly adsorbed by the particulate matter on the filter from previous puffs. The obvious means of minimizing this complication, changing the filter pad after each puff, is impractical when a large number of analyses are required on the entire deliveries of a large number of cigarettes. In our work, filter pads are changed after each cigarette as a compromise between necessity and practicality. Even with this precaution, as much as 50% of the hydrogen cyanide and an undetermined percentage of formaldehyde is lost (10) to the filter pad. Such losses are acceptable only because the biological assays of the gas phase also suffer these losses. An added concern from the analytical chemists' view point is that changing filters several times in the course of generating the sample might easily introduce an additional error point.

Progress made in establishing the gas phase analysis portion of this facility is summarized below.

4.01 ACETALDEHYDE AND ACROLEIN

A cryothermal gas chromatograph has been used with a smoking machine and tandem gas sample valves to determine acetaldehyde and acrolein in the gas phase of cigarette smoke. The goal of this development was to obtain complete separation of acetaldehyde and acrolein from all other components in the gas phase, and to accomplish this in the shortest possible time consistent with good recovery and good reproducibility.

The system (Fig. 2-2, 2-3, 4-1) for the analysis of the gas phase of cigarette smoke consists of three sections: 1. The ORNL smoking machine (Section 2.04); 2. Pneumatically operated tandem gas sample valves; and 3. The Microtek-150 gas chromatograph with the CT-75 cryothermal system. The gas phase sample from the smoking machine is directed to tandem gas sample valves. These pneumatic valves (Fig. 4-1) can be set in three modes: 1. Gas phase sample to column one, carrier direct to column two; 2. Sample to column two, carrier direct to column one; and 3. Sample from external source to a calibrated loop and, thence, to column one.

The Microtek-150 gas chromatograph is a dual-column instrument with a dual-flame ionization detector and a thermal conductivity detector. The control of the CT-75 cryothermal unit operates a solenoid valve that feeds liquid nitrogen to the column oven. The solenoid valve admits pressurizing gas, usually helium, to a 50-L liquid nitrogen flask. There are two modes of operation of the solenoid valve: wide open for fast cooling or pulsing for regulated cooling. The cryothermal system operates together with the column heating system to control the temperature of the oven. The oven can be cooled to -150°C and the temperature programming rate can be as little as 1° per min or as much as 40° per min.

The column used for the determination of acetaldehyde and acrolein in cigarette smoke consisted of 4-feet of 1/4-in copper tubing with a wall thickness of 0.035-in, packed with Burrell UCON in series with a column of the same dimensions packed with 20 wt.% β - β oxydipropionitrile on a 30-to-60 mesh acid-washed firebrick. An example of the separation produced by this column system is given in Fig. 4-2. Recent work has produced a new column system, Carbowax 200 and oxydipropionitrile, which appears to yield a peak for acrolein completely free of significant

interference. An example of the separation generated by this new column system is given in Fig. 4-3.

In operation, the column was held at -75°C and the carrier gas flow rate was set at approximately 70 cc per minute. The gas phase of the cigarette being analyzed was drawn into a gas-sampling loop during the puff and the content of the loop was swept by the carrier gas onto the cold column between puffs. When smoking was completed, the column temperature programmer warmed the column at a selected rate until acetaldehyde and acrolein eluted. A hydrogen flame ionization detector monitored the effluent of the column.

Results of the analysis of the experimental cigarettes using this technique are given in Section 10.00 of this report. The procedure allows these determinations to be carried out with exceptionally good precision (relative standard deviations of 2.5% and 4.0% for acetaldehyde and acrolein, respectively). The absolute values found are in general agreement with those reported elsewhere (14, 15, 30).

The acrolein peak generated by the Burrell UCON-oxydipropionitrile column contains a contribution from methylacetate. An average value of 10 micrograms per cigarette has been reported (33) for methylacetate. Under the flame detector conditions employed in these analysis, the molar response of methylacetate was determined to be 63% of the acrolein response. Therefore, the acrolein equivalent of methylacetate is only 6 micrograms. This is approximately 7.5% of the maximum value obtained for acrolein. It has been observed that the acetaldehyde-acrolein ratio remained relatively constant over the 23 experimental cigarettes and that the sizes of all the peaks diminish as the acetaldehyde and acrolein peaks become smaller. It is thus reasonable to expect that the methylacetate contribution to the acrolein value would be relatively constant.

It is because we cannot be certain that the methylacetate contribution is constant that additional effort was expended to resolve acrolein from methylacetate. A few analyses with the new column system should provide the data needed to assess the significance of this interference and determine the necessity of repeating the analyses for acrolein.

Collecting the entire gas phase from one cigarette at the head of a cold GC column has some advantages over collecting the sample in a cold trap (14, 15) and transferring it to the GC column. The residence time in the transfer lines is much shorter than with the trapping system and the desired components are completely trapped as an immobile "plug" on the head of the column. Also, a cryothermally programmed column allows the use of a much shorter column, and a shorter elution time. The chromatograms illustrated in Fig. 4-2 and 4-3 are intentionally "stretched out" to show the possibility of identifying gas phase components in the medium boiling range. The actual time of analysis according to the procedure given in this presentation is 1 hour and 10 minutes including oven cooling time.

An advantage is gained, also, by using a flame ionization detector rather than a thermal conductivity detector. Although the acrolein content of the gas phase is only about 5% that of acetaldehyde, the molar response of acrolein was determined to be four times that of acetaldehyde. This makes possible a more accurate measurement of the acrolein value.

The combination of a four-port smoker and cryothermal chromatograph yields two unique advantages. All four ports of the smoking machine could be operated (rather than one as was required in these determinations) and the combined smokes from any given puff or part of a puff could be analyzed. This should produce a more representative single-puff sample, and more precise single-puff data. Also, the ability to directly inject compounds onto the head of the column containing the plug of gas phase sample allows the use of quantitative internal standard techniques and could provide an accurate means of determining the retention indices (7) of the smoke components.

Three points remain of concern to this group: 1. Whether the peak for acrolein is truly free of interferences even with the new system, 2. Whether any acetaldehyde and/or acrolein might be lost due to condensation or adsorption during transit from the gas sampling loop to the column, and 3. Whether the acetaldehyde and/or acrolein might react while being held at the head of the column. The first concern can only be answered by monitoring the peaks with a mass spectrometer but none is

available for this application. The second and third concerns may be studied using ^{14}C -labelled acetaldehyde and acrolein.

The procedure used for the determination of acetaldehyde and acrolein in the sample cigarettes is detailed in Section 9.05.

4.02 CARBON MONOXIDE AND CARBON DIOXIDE

The procedure being established here for the determination of carbon monoxide and carbon dioxide in the gas phase of smokes has not been completely tested. Since it is a proven (compliments of R.J. Reynolds Research) procedure involving straight-forward techniques, we anticipate no difficulty in applying it in a routine manner.

The gas phase of the cigarette is routed to an evacuated rubber balloon that is attached to the exhaust side of the syringe of smoking machine No. 3 (Section 2.05). The adapter joining the smoking machine to the balloon is fitted with a rubber septum through which the contents of the balloon is sampled using a gas-tight syringe. Samples are simply taken and injected onto a gas chromatography column.

A six-foot by 1/4-in o.d. column of Porapak Q operated at approximately 75°C with a 30 cc/min helium carrier gas flow rate gives an adequate separation of air plus methane plus carbon monoxide from carbon dioxide. A three-foot x 1/4-in o.d. column of molecular sieve-5A operated at approximately 75°C with a 70 cc/min helium carrier gas flow rate separates carbon monoxide from oxygen, nitrogen and methane. Both columns are connected to the thermal conductivity cell of the Microtek-150 gas chromatograph. Total analysis time after smoking the cigarette is anticipated to be less than 10 minutes.

The procedure is outlined in Section 9.06.

4.03 FORMALDEHYDE

The procedure for the determination of formaldehyde in the gas phase of smokes recommended by the Tobacco Working Group utilizes an aqueous chromotropic acid solution as an absorber. A single puff of smoke is drawn through a Cambridge filter into a glass bulb containing 5 ml of the absorbing solution. After a short contact time, the solution is drained from the bulb, treated, and analyzed spectrophotometrically.

Our need to analyze the total delivery of each cigarette rather

than that of a given puff as well as the relatively large number of samples to be examined necessitated a search for a dynamic trapping system. The trap diagrammed in Fig. 4-4 and pictured in Fig. 4-5, suggested by workers at R. J. Reynolds Research, was the result of this search. A good color development was noted with standard formaldehyde solutions using the method described by Newsome (20). No formaldehyde could be detected in the smokes generated here when the standard colorimetric procedure was applied to the contents of the adopted traps. In agreement with the information supplied us by Iyerly (16), we found that the incorporation of sodium metabisulfite in the trap yielded meaningful formaldehyde data. The overall determination remains essentially as described by Newsome (20). Table 4-1 summarizes the results obtained when this procedure was applied to a commercial cigarette and the Kentucky Reference Cigarette. Precision is reasonable. The amount of formaldehyde found is in general agreement with the amount expected to be present (20,33).

The use of chromotropic acid for the detection of formaldehyde seems to be reliable. Its color complex remains stable for at least two hours. However, a standard formaldehyde solution should be examined with each set of samples to ascertain the reliability of the standard curve. The fact that this reagent is not specific does not appear to be a serious disadvantage as long as a base-line absorbance reading is noted and the correction is applied.

The procedure in use for the routine determination of the formaldehyde deliveries of the experimental cigarettes is detailed in Section 9.08 of this report.

4.04 OXIDES OF NITROGEN

The initial recommendations of the Tobacco Working Group referred to a colorimetric procedure (20) which utilized the Saltzman reagent as an absorber for the oxides of nitrogen in cigarette smoke. For this determination, a puff of smoke is drawn through a Cambridge Filter into a 55 ml bulb containing 10 ml of the Saltzman reagent. After allowing an hour for air oxidation of NO to NO₂ and full color development, the solution is removed from the bulb and measured spectrophotometrically.

Good color development was achieved by the reagent with standard nitrite solutions. When the reagent was employed as an absorbing solution in our dynamic trap (that used for formaldehyde collection, Section 4.03), less than 15 μg or about 5% of the amount expected was found. It is apparent that insufficient contact-time is available with such a system for the absorption of NO and air oxidation of NO to NO₂. A series of three Nesbitt bubblers, each containing 50 ml of trapping solution and an inch layer of glass beads, proved to be a superior trapping system. A total of 137 $\mu\text{g}/\text{cig}$ of oxides of nitrogen was found using such a system. However, this is well below the 200-300 $\mu\text{g}/\text{cig}$ expected (25) to be present. A series of Milligan bubblers appeared to provide superior gas-liquid contact, but resulted in an excessive back pressure during the puffs. These bubblers were not studied further because of a fear of altered smoking characteristics. Smoking machine number 2 (Section 2.04) was used in a final attempt to develop a satisfactory dynamic trapping system. Smoke was drawn into the gas sampling loop, and flushed between puffs through a series of three fritted gas dispersion bottles. Analyses of the traps yielded only 51 μg of NO₂. The direct approach of drawing the smoke through the dispersion bottles was found equally unsatisfactory. It appears that the Saltzman technique is not applicable using dynamic trapping. The smoke could be sampled by individual puffs and the delivery of oxides of nitrogen determined by summing the results for each puff. The labor involved with such a technique precludes its use as a routine method for large numbers of cigarettes.

Discussions with Dr. G. Morie of Tennessee Eastman have resulted in a promising alternative trapping technique. The smoke is drawn into the syringe of smoking machine number 3 (Section 2.05) and exhausted into an evacuated bulb containing the Saltzman reagent. A slight modification of the smoking machine is necessary before this technique can be tested. Experience with the colorimetric procedure and single-puff collection suggests that this new approach will be successful. Once proven, this technique will be used for the routine analyses.

A completely different approach to the determination of oxides of nitrogen has been provided to this group by the staff of R. J. Reynolds Research. In this procedure, the smoke is collected in a trap (Fig. 4-4) containing pyridine, dimethylbenzylamine and water. The NO_2 content of the traps is determined by non-aqueous titration. Both potentiometric and visual end-points have been used in the study of this procedure. Typical results are given in Table 4-2. Reasonable values are generated with acceptable precision. This procedure serves as a valuable alternative to the more standard Saltzman procedure.

The method of Barkemeyer and Seehofer (4) has been considered and temporarily rejected because of the large sample required (45 cigarettes for a triplicate analysis). The state-of-the-art method described by Urbanic (32) for the direct determination of NO in the gas phase of cigarette smoke is of great interest, but has been temporarily rejected because of the unavailability of suitable equipment. A procedure, the peroxide oxidation of NO to NO_3^- followed by ion-selective electrode measurement of NO_3^- , developed in-house requires substantial additional development before it could serve as a reliable method.

The procedure expected to be used in the routine analysis of the experimental cigarettes is detailed in Section 9.07 of this report.

4.05 HYDROGEN CYANIDE

The electrometric method of Baker and Morrison (2) described by Newsome (20) has been investigated at length and rejected as unreliable in our hands. We found the technique to be greatly influenced by experimental conditions, and we have experienced extraordinary difficulty in generating a reliable calibration curve.

Methods generated in-house for the determination of hydrogen cyanide appear promising. In one case, the cyanide content of the trap is determined polarographically while in another case, a cyanide ion-selective electrode is employed for the determination. The ion-selective electrode procedure has consistently produced higher than anticipated results, but shows particular promise. Neither method is sufficiently proven to justify its application in these initial analyses.

A procedure for the determination of hydrogen cyanide has also been provided by workers at R. J. Reynolds for our consideration. This procedure involves the trapping of hydrogen cyanide in an appropriate solution contained in our standard (Fig. 4-4) trap, and the subsequent spectrophotometric determination of cyanide by the method of Schilt (28). We have found the technique to produce reasonable values with acceptable precision. The single disadvantage is that the procedure is relatively time consuming.

A more convenient means of determining the hydrogen cyanide delivery of cigarettes involves trapping the gas phase component on Ascarite (5, 17), and a colorimetric (1, 3) measurement of the extract. Results obtained with this method compare favorably with those obtained using the procedure of Schilt. Table 4-3 summarizes typical results. This procedure is presently in use for the quantitative determination of the hydrogen cyanide deliveries of the experimental cigarettes. Details are given in Section 9.09 of this report.

4.06 GAS PHASE pH

An empirical method for measuring the pH of cigarette smoke has been reported by Sensabaugh and Cundiff (27). The method has been tested here and appears to be satisfactorily adaptable to the determination of the pH of the gas phase of smoke.

A picture of the assembly employed for this measurement is presented in Fig. 2-4. Smoke is drawn across the tip of a modified combination pH electrode. The response of the electrode is monitored continuously by a Heath electrometer strip-chart recorder. Applications of this technique to whole smoke yield curves essentially like those reported by Sensabaugh and Cundiff (27). Substantial differences in response are visualized when the technique is applied to the gas phase. The span of the recorder deflection (the change in pH) is generally smaller during a given puff than is observed for whole smoke. The deflection during a puff is often in the opposite direction than for whole smoke. The direction of the deflection is often different for the early puffs than later puffs. Particularly striking is the fact the difference between the pH observed for the second puff and that for the last puff is often two to three

times as great as that observed for whole smoke. The fact that each succeeding puff is drawn through a filter pad containing successively greater amounts of particulate matter undoubtedly contributes to the unexpected pH patterns. A meaningful interpretation of the patterns will be difficult. The pH found at given puffs and the final pH may be of some value in a relative sense when the different experimental cigarettes are compared.

The procedure is detailed in Section 9.10 of this report.

5.00 ANALYSIS OF THE CONDENSATE

Cigarette smoke condensate is generally prepared by drawing standard puffs of smoke through cold traps maintained at from liquid nitrogen to dry ice temperatures. Condensates represent the most complete samples of smokes - only permanent gases are regularly lost in the condensation procedure. Many high vapor pressure components are undoubtedly lost in subsequent steps of dissolution and concentration during the preparation of condensate samples. Opportunities for the chemical constituents of the condensates to react with one another, though impossible to eliminate, are lessened by keeping the concentrates cold and avoiding excessive exposure to air and light.

Biological testing of the prepared condensates is the foundation of studies of experimental tobacco carcinogenesis. Chemical analyses of the condensates provides data to both foster and test theories of chemical carcinogenesis. The production of representative and reproducible condensate samples requires frequent tests of the chemical compositions of the respective preparations.

A summary of the progress made in establishing the capabilities of analyzing the smoke condensates of the experimental cigarettes in a routine operation is given below.

5.01 SAMPLING PREPARED CONDENSATE

Samples of condensate received at this laboratory have a composition of approximately 65% condensate, 25% acetone, and 10% water. It is difficult, yet critical, to withdraw aliquots representative of the entire sample and to do so reproducibly. No standard sampling procedure has been defined.

More than 30 samples have been withdrawn for various studies from a test quantity of 1R1 condensate prepared at Melabs. Experience to date indicates that reproducible sample weights can be aliquoted by sampling the condensate suspension with a transfer pipette while the condensate is stirred with a magnetic stirrer. The content of the pipette is quickly discharged into a vial. The vial is capped and the sample size is determined by weight.

Sampling by transfer pipette is reproducible but may not result in representative samples. We note a somewhat higher result for the percentage of nicotine when large samples were analyzed than for smaller samples. More vigorous and more random agitation of the condensate than can be obtained using a magnetic stirrer will be required to produce a homogeneous suspension. This methodology will be studied further using determinations of the "phenol number" (21) and total alkaloids (22) as measures of sample homogeneity resulting from various sampling procedures.

5.02 WATER AND ACETONE

The final condensate preparations are adjusted to a composition of approximately 65% condensate, 25% acetone, and 10% water by the addition of water and/or acetone as needed. It is necessary to know the precise composition of the final preparation in order to plan the dosages needed in biological testing and to interpret the results of such testing. Analyses for acetone and water are carried out regularly at Melabs to control the production of condensate preparations.

Analyses for acetone and water are also carried out at ORNL on the condensate preparations submitted for chemical testing. The results generated here constitute a cross-check between Melabs and ORNL. The results may also reveal the influence of shipping and storage delays on the gross compositions of the preparations. Most important, the quantity of dry condensate in the particular fraction undergoing chemical testing must be known to establish sample sizes to be taken and to allow expressing results on a dry condensate basis.

A gas chromatographic column of 80-100 mesh Porapak Q is used to separate acetone, water and an internal standard. Ethanol has been selected as the best internal standard for use with Porapak Q because it elutes between water and acetone (Fig. 3-1). The use of a column three feet long at a temperature of approximately 180°C allows the separation to be completed in about six minutes.

In practice, a sample of approximately 100 mg. of condensate is contacted with two milliliters of 100:1 dioxane:ethanol, the solution

is mixed and about 5 μ l of the sample is injected into the chromatograph. The relative peak heights, water:ethanol and acetone:ethanol, are determined and compared to a calibration curve. Preliminary studies indicate that the procedure is capable of producing results with relative standard deviations of 2.5% and 3.5% for acetone and water, respectively, in a given condensate preparation. A comparison of the results obtained here with those reported by Melabs for a given preparation indicates that the procedure used here yields reasonable results.

The procedure in use is outlined in Section 9.11.

5.03 NICOTINE AND NORNICOTINE

Both the spectrophotometric method of Ogg (22) and a gas chromatographic method comparable to that of Mumpower and Kiefer (19) will be used in early analyses of condensates to assess their contents of nicotine alkaloids. The spectrophotometric procedure is the most convenient, but yields a measure of total alkaloids without distinguishing nicotine from the minor alkaloids. The spectrophotometric procedure is essentially the same as that used for the analysis of particulate matter (Section 3.03). The gas chromatographic procedure can provide distinct values for nicotine and nornicotine, but will not detect other alkaloids without modification. A comparison of the results from each method will define the preferred assay system and limitations of each.

The work of Mumpower and Kiefer has demonstrated that sufficient nicotine exists in smoke to allow the direct analysis of extracts of whole particulate matter without prior purification and concentration. Using a comparable casterwax column, we make the same observation for condensate preparations. The separation achieved with a casterwax column is demonstrated in Fig. 5-1.

The direct gas chromatography of solutions of whole condensate represents a major improvement in convenience over methodologies requiring steam distillation and liquid-liquid extraction. Because the entire sample is analyzed, there is no need to employ carbon-14 techniques to monitor the recovery of nicotine. Methods involving purification, concentration and counting may be necessary for the analysis of condensates

from low nicotine-delivery cigarettes. The facilities necessary to employ such a method are readied in the event of need.

The direct gas chromatography procedure involves contacting a sample of condensate with methanol containing dimethylphthalate as an internal standard. The ratios of the peak heights of nicotine:dimethylphthalate and nornicotine:dimethylphthalate are simply compared to a calibration curve to determine the nicotine and nornicotine contents of the sample. The column system works well for the detection of nicotine, but has behaved erratically in the detection of nornicotine. A. R. Patel (23) reports a similar observation and recommends the use of neopentyl glycol adipate for the simultaneous visualization of nicotine and nornicotine. Either a concentration procedure or a more efficient column than castorwax may be necessary to carry out the simultaneous determination of nicotine and nornicotine.

A general procedure is outlined in Section 9.12.

5.04 PHENOL AND THE CRESOLS

Two assay systems are expected to be employed for the measurement of phenols in condensate: a non-specific colorimetric procedure (21) to determine the homogeneity and stability of the samples, and a gas chromatographic procedure to determine the phenol, o-cresol, and m+p-cresol contents of the samples. In the colorimetric method, phenols are steam-distilled from acidified condensate and reacted with 4-aminoantipyrene. Some selectivity is achieved by extraction of the products into chloroform before measurement, but the result remains only a "phenol number".

Two methods of preparing samples for gas chromatographic analysis have been tested thus far. The procedure outlined (33) in the recommendations of the Tobacco Working Group, involving extractions, concentrations, steam distillation, extraction and final concentration, has been found of questionable reliability in our hands. A carbon-14 tracer experiment indicated recoveries of phenol as low as 50%. A less involved procedure (21), requiring only two extractions, has been found suitable for the preparation of phenol fractions for gas chromatographic analysis. Recoveries of better than 80% have been observed for phenol taken through

this procedure. Although no provision is made to separate the phenols from the stronger acids, we have observed no responses in the chromatograms to indicate that these acids interfere in the analysis.

Studies (Section 6.05) of the gas chromatography of phenols helped define suitable chromatographic conditions. Casterwax (Fig. 5-2) proved to rank among the most suitable phases for this analysis when resolution, retention, and sensitivity were considered. Di-n-octylsebacate columns prepared here allowed considerable overlaps of guaiacol with phenol as well as o-hydroxyacetophenone and 2,6-xyleneol with o-cresol when the columns were operated isothermally. Under programmed temperature operation, the only resolution problem was with o-cresol and 2,6-xyleneol. The primary difficulty with the use of di-n-octylsebacate is that temperatures well above the maximum recommended levels must be used to elute the phenols in reasonable times. This results in excessive column bleed and limits the column to low-sensitivity applications.

A number of other separating phases have been examined for this application. Neopentyl glycol sebacate (NGS) was examined as a phase similar to di-n-octylsebacate, but with better thermal properties. NGS did not adequately resolve o-hydroxyacetophenone from phenol nor 2,6-xyleneol from o-cresol. Trixylol phosphate columns have been found to completely resolve phenol and the cresols from those phenols (salicylaldehyde, gualicol, o-hydroxyacetophenone, and 2,6-xyleneol) which commonly interfere. Only 2,4,6-trimethylphenol emerged in the phenol-cresol range. Unfortunately, we have found it particularly difficult to reproduce these separations with new preparations of trixylol phosphate columns. Methylsilicones such as OV-101 do not resolve salicylaldehyde from o-cresol. Polyhydroxy phases such as carbowax 20M and FFAP and polyester phases such as ethylene glycol phthalate produce very poor separations of phenol and the cresols from a large number of smoke-related phenols. Of all the phases studied, only the trixylol phosphate column produced distinct peaks for m-cresol and p-cresol. Resolution was far from complete, however.

A preliminary procedure for the determination of the phenols in condensate is outlined in Section 9.14.

5.05 PHENANTHRENE, BENZ(A)ANTHRACENE, AND BENZO(A)PYRENE

The quantitative isolation and measurement of single polycyclic aromatic hydrocarbons, as components of cigarette smoke, is begun by extracting the polycyclics from methanol-water suspensions of smoke condensate with cyclohexane. Most of the more polar components are left in the suspension while the bulk of the neutral components enter the cyclohexane. Fractions enriched in polycyclics are next obtained by extracting the cyclohexane with nitromethane or dimethylsulfoxide. When dimethylsulfoxide (DMSO) is used, it is simply contacted with an excess of water and the polycyclics are back-extracted into cyclohexane. Nitromethane extracts must be subjected to complete evaporation and the residue dissolved in hexane or cyclohexane. The hexane or cyclohexane solutions are then concentrated by carefully evaporating the solvent. The result of the extractions is a small volume of the paraffinic solvent containing the bulk of the neutral aromatic components of the condensate.

The aromatic concentrate is next transferred to an alumina column and eluted with hexane and hexane-benzene mixtures. The effluent is collected in fractions and the fractions examined for distribution and purity of polycyclics by fluorescence measurements and carbon-14 tracer scintillation counting. (Any practical quantitative method of this complexity necessitates the addition of labelled counterparts of the components of interest at the start of the analysis in order to access the recovery of those components). The fractions containing phenanthrene, benz(a)anthracene (BaA) and benzo(a)pyrene (BaP) are composited and each is concentrated to a volume of approximately 0.5 ml. The concentrates, enriched in the specific compounds of interest, are ready for final purification.

Each concentrate is transferred onto a thin-layer chromatography plate or paper chromatography sheet and chromatographed using a mixture of methanol-ether-water. The developed chromatograms are examined under ultraviolet light, the bands of interest are marked and cut out, extracted or eluted with benzene-alcohol, and the extracts are gently taken to dryness under a nitrogen atmosphere. The residue is redissolved in cyclohexane, freed of lint and other particulates by alumina column filtration,

and diluted to volume. The end result is three fractions prepared for measurement. Aliquots of the final preparations are taken for carbon-14 counting and quantitative fluorescence measurement.

The phenanthrene fraction may be analyzed by gas chromatography if a column capable of resolving anthracene from phenanthrene is available. The gas chromatographic method may allow the paper chromatographic step to be omitted for phenanthrene. In order to minimize reactions of the polycyclics during the sample preparation, all evaporations are carried out with a gentle stream of nitrogen. A water bath maintained at 45°C is used when needed. All work is carried out in laboratories equipped with yellow lights.

The overall procedure has not yet been tested at this laboratory to our satisfaction. The efficiency of the various stages of the procedure are presently being examined using tracer techniques. Progress in the various stages of the procedure is outlined below. A preliminary detailed procedure is presented in Section 9.16 of this report.

A. Extractions. Cyclohexane appeared to function well in the removal of the polycyclics from the methanol-water suspension of the condensate without first evaporating the acetone. An additional experiment will be carried out to determine whether the prior evaporation of acetone provides a superior recovery of polycyclics. Nitromethane has been confirmed to be a satisfactory extractant for the preparation of polycyclic-enriched neutral extracts. The time required to completely evaporate the nitromethane and, particularly, difficulties experienced in redissolving the residue suggest that the use of nitromethane is less than ideal. Dimethylsulfoxide (Section 6.0) has been found to be a promising alternative to nitromethane.

B. Column Chromatography. Several adsorbents other than alumina and an analytical liquid chromatograph have been evaluated (Section 6.0) as possible improvements over the recommended (33) chromatographic procedure. The use of alumina with hexane and hexane-benzene eluents, as recommended, remains the most practical procedure. Separations of phenanthrene, BaA, and BaP have been accomplished in less than four hours with the alumina column technique. Phenanthrene is retained long enough

to allow the prior elution of the bulk of the less active components of the fraction and is well resolved from BaA. Baseline resolution of BaA and BaP has not yet been observed. Studies of the recoveries of phenanthrene and BaA from an alumina column showed that 99% of that added to the column will be found in the effluent. Labelled BaP was not available at the time of this study.

C. Paper Chromatography. Sheets of acetylated cellulose paper (20 x 20 cm) were used with a developer of 4:4:1 methanol:ether:water for a study of the chromatographic characteristics of the polycyclics. Phenanthrene, BaA, and BaP were readily separated in less than three hours. Anthracene was not completely separated from phenanthrene. Comparable results were obtained by thin-layer chromatography on acetylated cellulose. The larger sample size usable with the paper chromatographic system makes this the preferred matrix at this time.

D. Measurements. Conditions for a convenient gas chromatographic separation of anthracene from phenanthrene are presently being sought. Standard solutions of the polycyclics have been examined with a Perkin-Elmer Model 203 fluorescence spectrophotometer to determine suitable excitation and emission conditions. Excellent calibration curves could be obtained for each polycyclic. Manually generated fluorescence spectra of the compounds agreed well with published spectra.

5.06 WEAK AND VERY WEAK ACIDS

A non-aqueous potentiometric titration with tetrabutylammonium hydroxide will be utilized to analyze the acid content of smoke condensates. The procedure selected for the analysis is that of Cundiff (6). A preliminary investigation of the technique and apparatus involved in this determination has been completed. Tetrabutylammonium hydroxide titrations of mixtures containing hydrochloric acid, 3,5-dinitrosalicylic acid and benzoic acid in CO₂-free pyridine yield three good potentiometric inflection points which agree well with the known concentrations for each acid. Standard solutions of 3,5-dinitrosalicylic acid and benzoic acid must be added to pyridine solutions of the condensate to produce usable titration data. The quantity of acid present in the condensate can then be determined by difference.

It is imperative to protect the titration vessel from contact with air. Carbon dioxide is readily absorbed by the pyridine and is titrated as an acid. Maintaining a blanket of argon over the sample during titration and protecting the titrant with an ascarite trap provides sufficient protection from carbon dioxide interference.

The influence of acetone and the condensate on the titration remains to be determined. A tentative procedure for the titrimetric determination of acids in condensate is given in Section 9.7 of this report.

5.07 pH OF CONDENSATE

Additional development work is necessary to provide a means of obtaining a meaningful value for the pH of condensate. A relative measure of the pH can be obtained by contacting samples with sufficient CO_2 -free water to yield solutions of a given concentration of condensate and directly measuring the pH with a combination electrode. The "pH" of the original sample might be back-calculated from the determined pH and known dilution.

The "pH" of the condensates are expected to be slightly acidic (pH 4-7). Care must be taken to avoid exposing the extracting water to the air as absorbed CO_2 can greatly influence the pH reading. Plans are to carry out dilutions and the pH measurement in an inert gas atmosphere. The pH of the extracting solvent must be examined daily.

A tentative procedure is outline in Section 9.18 of this report.

6.00 RESEARCH ACTIVITIES

The bulk of research-related activities of this effort have been of a development nature in direct support of the routine operations. These activities have been discussed in the preceding sections of this report. This section presents brief summaries of those research activities of particular interest to this group though not necessarily related to the routine operations.

A substantial portion of the basic research carried out thus far is related to component profiling. "Component Profiling" used in this sense refers to chromatographically displaying sub-fractions of smoke or smoke condensate. The peaks in such chromatograms constitute a profile of the chemical components of the sample. Such chromatograms would be expected to allow the simultaneous quantitative determination of large numbers of closely related chemical smoke components in single analyses. The relative quantities of large numbers of components could also be determined for given samples of smoke or condensate. Such procedures could allow the immediate detection of unsuspected differences in the chemical composition of samples of smoke or condensate from different sources or following different treatments.

No effort has yet been expended to produce high resolution chromatographic component profiles. The primary concern to this point has been to produce assay procedures which involve a minimum of specialty technique and specialty hardware, and can be employed by workers other than chromatographic specialists. Extending these capabilities into the high resolution field would be an obvious next step should the profiling technique prove to be significantly worthwhile. The work of Grob (12) clearly defines the approach to higher-resolution methodologies.

6.01 N-NITROSAMINES

A preliminary investigation has been made to determine the feasibility of identification and/or determination of N-nitrosamines in cigarette smoke by a polarographic method. The nitroso group is reduced at the D.M.E. in several base electrolytes.

Heath and Jarvis (13) have determined N-nitrosodimethylamine polarographically using 0.01 M sulfosalicylic acid as a base electrolyte. We found the N-nitrosoamines to be stable for at least several hours in this solution, but better formed reduction waves and more reproducible diffusion currents were obtained when the concentration of the sulfosalicylic acid was increased to 0.1 M. It was also necessary to add methanol to the base electrolyte solution to increase the solubility of the higher molecular weight compounds. The final base electrolyte solution selected was 0.1 M sulfosalicylic acid, 10% methanol, and 90% water. Polarograms were obtained in this solution for the six compounds listed in Table 6-1 using a drop of 0.5 second, a scan rate of 1 v/min, and a potential range of 0 to -1.2 v vs the S.C.E.

Well formed waves were obtained for five of the compounds. The half-wave potential appears to be related to the molecular weight with the higher molecular weight compounds being more easily reduced. The solutions contained 20 µg/ml of the nitrosoamine compound in all cases, except for N-nitrosodimethylamine. To estimate the detection limit, the concentration of N-nitrosodimethylamine was varied from 20 to 0.2 µg/ml. Well formed polarograms were obtained at a concentration of 0.2 µg/ml, and it is estimated that 0.04 µg/ml could be detected by first derivative polarography.

One commercial cigarette was smoked through a polarographic cell containing 5 ml of the base electrolyte solution. A reduction wave with a half-wave potential of approximately -0.54 v was obtained; however, the wave was very broad indicating either a mixture of many compounds, or a very irreversible reduction process. Further work, separating the smoke or smoke condensate into fractions, would perhaps show whether the wave is due to a mixture of several compounds or not.

6.02 GAS CHROMATOGRAPHIC COMPONENT PROFILES - WHOLE SMOKE CONDENSATE

A rapid, simple assay of whole condensate for its major chemical constituents is highly desirable. The direct injection of condensate or condensate suspensions into a gas chromatograph has proved to be a marginally useful approach. The chemical complexity of condensate results in a badly wandering baseline, apparently due to extreme overlap and poor

chromatographability of many major constituents. Quantitative reproducibility of the profiles is very poor - due both to poor chromatographability and sampling difficulty.

A study of the utility of small-volume extraction or dissolution followed by trimethylsilylation suggests this to be a very useful approach to whole condensate profiling. The dissolution of condensate in pyridine and subsequent silylation allows the generation of gas chromatographic profiles of whole condensate which display at least forty of the major chemical components of the condensate. Such profiles (Fig. 6-1) can be generated in less than an hour. The entire procedure requires less than 50 mg of condensate.

The finding that pyridine allows the complete dissolution of acetone-water suspensions of condensate suggests means for better precision in sampling, and for the use of internal standards. The fact that pyridine is an ideal solvent for silylation makes the procedure particularly convenient. The water content of the condensate suspensions (~10% by weight) has had no detectable influence on the silylation procedure.

Experiments have been carried out to provide a measure of the quantitative reproducibility of the chromatograms over replicate injections of given samples and from sample to sample. Preliminary studies of the influence of concentration procedures, light, temperature, and time on the appearance of the chromatograms (profiles) have also been carried out. Preliminary small volume extraction of the condensate into acid or base followed by profiling the ether extract has revealed the essentially acidic nature of the majority of the visualized components.

The primary conclusion of these studies thus far is that the profiles can be generated in a very reproducible manner. As such, the technique may provide a particularly convenient means of detecting gross differences in the compositions of the condensates.

6.03 GAS CHROMATOGRAPHIC COMPONENT PROFILES -GAS PHASE OF SMOKE

The chromatograms given in Figs. 4-2 and 4-3 constitute gas chromatographic profiles of a small range of components in the gas phase of smoke. Chromatograms like that of Fig. 4-2 were generated for each experimental cigarette in the course of the determinations of acetaldehyde

and acrolein. Generally, the relative quantities of the various components in these profiles remained relatively constant from sample to sample. Some cigarettes did produce substantial relative differences for some components of the profiles. The data are in need of further study before meaningful conclusions can be drawn.

The range of compounds visualized in gas phase profiles can easily be extended (12). The availability of the cryothermal system suggests that an apparatus can be designed to allow an extremely large number of species, including inorganic gases and organic components, to be visualized. Such a system could provide a unique opportunity to characterize the overall composition of the gas phases of the experimental cigarettes on a puff by puff and total delivery basis.

6.04 SULFUR-CONTAINING COMPOUNDS OF SMOKE

The direct application of flame photometric detection and porous-polymer column gas chromatography to the study of the gas phase of cigarette smoke provides an excellent means of elucidating the composition of the sulfur-containing portion of smokes. It was necessary to employ a manually operated syringe to "smoke" the cigarettes for the preliminary work which does not allow the generation of meaningful quantitative data. Cigarettes were smoked through Cambridge filters, the filter holder and cigarette removed from the syringe, all but 5 of the 35 cc of gas phase was exhausted from the syringe, a needle was quickly attached and the contents of the syringe was injected into the chromatograph. In spite of these manipulations, reproducibility (Fig. 6-2) was good enough to draw preliminary conclusions about the levels of sulfur compounds in smokes.

Our results indicate that there may be more H_2S than COS in cigarette smoke. This is in direct contrast to the data presented by others (33) which indicates the presence of five times as much COS as H_2S in cigarette smokes. Recently published (31) data suggest the presence of comparable levels of H_2S and SO_2 in smokes. We have not been able to detect any SO_2 at 100 times the sensitivity which easily visualizes H_2S . Also of interest is the fact that we observe at least 14 sulfur-bearing species with this single system. Only ten sulfur-containing species were reported in the review by Stedman (26).

The sensitivity of the technique is such that 50 μ l of the gas phase of smokes is sufficient for quantitative determinations to be made. This suggests the possibility of designing experiments that will allow studies of smoke-aging phenomena in smokes approximately 100 msec old. No technique is available at present which allows studies of smokes this fresh using a chemical marker.

The technique has been applied to a survey of the H_2S -COS deliveries of commercial filtered cigarettes (Fig. 6-3 and 6-4). Cellulose acetate filters were found to provide a slight overall reduction in delivery of sulfur species. The influence of charcoal filters was quite dramatic. Not only did charcoal filters provide substantial reductions, but the filtration was also highly selective. Hydrogen sulfide was removed substantially better than was COS. The design of the filters may also be important— granular charcoal appeared to be more effective than fibrous charcoal.

A more complete display of the sulfur delivery of cigarettes is given in Fig. 6-5. The similarity between the Kentucky Reference Cigarette and the commercial charcoal filtered cigarette with the filter removed is striking. The ability of the charcoal filter to effect a reduction in the sulfur delivery is also apparent in Fig. 6-5.

6.05 PHENOLS OF CIGARETTE SMOKE

The phenolic sub-fraction of smoke condensate contains a large number of weakly acidic components that might be visualized by a component profiling technique. Considerable effort has been expended in studies of gas chromatographic methodologies which will allow the visualization of the maximum number of smoke phenols in a minimum of time. It was during these studies that the observations of potential interfering phenols in the standard phenol-cresol procedure were made (Section 5.04).

Approximately 30 smoke-related phenols have been chromatographed using ten different chromatographic systems. Included among the separating phases studied are Carbowax 20M, FFAP, Ethylene Glycol Phthalate, Neopentyl Glycol Sebacate, Cyclohexanedimethanol Succinate, Di-n-octylsebacate, Castorwax, Trixyloolphosphate, OV-101, and OV-17. Examples of the separations produced by four of these systems are given in Fig. 6-6, 6-7, 6-8, and 6-9 for the phenols in Table 6-2.

None of the tested phases resolved all of the phenols of interest. Polyhydroxy phases such as Carbowax 20 M (Fig. 6-6) produced excellent peak definition but did not resolve phenol from o-cresol. Polyesters such as ethylene glycol phthalate resolved the phenol/o-cresol pair, but 2,6 xyleneol eluted with phenol. Polyesters were generally inferior to the polyhydroxy phases for resolving large numbers of phenols

Trioxylol phosphate (Fig. 6-7) provides a good separation of low molecular weight phenolics, but a poor separation of the higher molecular weight phenols. The relatively high vapor pressures of trioxylol phosphate and di-n-octyl sebacate make these phases inapplicable to the quantitative analysis of phenols which elute much later than the cresols. Casterwax (Fig. 6-8) provides a reasonable balance between resolution and range of applicability. Methyl silicones (Fig. 6-9) provide excellent peak definition, reasonable resolution and the widest range of applicability but allow salicylaldehyde to elute with phenol. Poor retention of the phenols by methyl silicones frequently causes interferences by the solvent when high sensitivities must be used.

Preliminary work has been carried out on means of preparing and concentrating phenol extracts. The addition of a small volume of a slightly miscible solvent of high vapor pressure as a scavenger prior to the evaporative concentration of ethereal phenol extracts greatly lessens losses of phenols during concentration. Continuous extraction techniques have been examined briefly and appear promising.

Preliminary work has been carried out to test the feasibility of distinguishing between phenols, acids and phenolic carboxylic acids by a double derivatization - multiple detection technique. A brief consideration of the compatibility of heptafluorobutyrylimidazole (HFBFI) and heptafluorobutyrate of monohydric phenols with trimethylsilylimidazole (TSIM) and bis-trimethylsilyl trifluoroacetamide (BSTFA) indicates that TSIM converts HFB-phenols to TMS-phenols. BSTFA appears to be more compatible with the HFB-phenols.

6.06 NITROGEN-CONTAINING COMPOUNDS OF SMOKE CONDENSATE

The chromatographic behavior of approximately forty smoke-related nitrogen-containing compounds has been established for four different

separating phases. Included among the compounds studied are alkylpyridines, alkyylanilines, alkylindoles, pyridine alkaloids, and miscellaneous aromatic nitrogen-heterocyclics. Not all of these compounds would be expected to occur in a given subfraction - some are basic while others are neutral. The possibility of visualizing nitrogen-containing species in a primary fraction using electrical conductivity detection requires that the chromatographic behavior of classes of nitrogen compounds be characterized for given column systems.

Carbowax 20 M, FFAP, Ethylene Glycol Phthalate and Cyclohexanedi-methanol Succinate (HiEFF) have been employed in these studies. A representative chromatogram is given in Fig. 6-10. The polyglycol columns are capable of resolving at least thirty-two smoke-related bases in approximately forty minutes. The same sample would generate only 22 peaks on HiEFF and 17 on thylene glycol phthalate. A single chromatogram will display substituted pyridines, anilines, and indoles using a polyglycol column, but polyester columns allow pyridines to elute too near the solvent.

6.07 CARBOXYLIC ACIDS OF SMOKE

Gas chromatography becomes difficult for free carboxylic acids much larger than butyric acid. Methylation results in excellently chromatographable derivatives, but is of questionable quantitative utility when applied to complicated mixtures of acids. Experiences with the silicone-specific detector (18) suggest that trimethylsilylation proceeds essentially quantitatively. Twenty-two of twenty-seven components of a synthetic sample of aliphatic and aromatic carboxylic acids were resolved in twenty minutes when chromatographed as trimethylsilyl esters.

6.08 POLYCYCLIC AROMATIC HYDROCARBONS OF SMOKE CONDENSATE

The analysis of condensates for the determination of polycyclic aromatic hydrocarbons is the most time-consuming and difficult analysis of this program. Although this analysis is among the most thoroughly researched in the tobacco sciences, many opportunities exist for its improvement. Brief summaries of our activities related to this problem follow.

An obvious improvement over the present methodology would be an approach which allows the determination of the polycyclics in the alumina

column fractions without requiring a paper chromatographic purification. Theoretically, electron capture gas chromatography of concentrates of the column fractions would provide the needed resolution and sensitivity. Capillary column gas chromatography may be necessary to achieve the required resolution. We find that the standard packed columns of OV-101 and OV-17 do not provide the needed resolution. Phenanthrene is not separated from anthracene, BaP and BeP are incompletely resolved and BaA is contaminated with chrysene when OV-101 is used.

A study of the chromatographic properties of more than 20 polycyclic aromatic hydrocarbons suggests that peaks for the components of interest will contain contributions from other polycyclics in addition to those mentioned. Neutral nitrogen-heterocyclics can also interfere.

Advantage may be taken of the relative sensitivities of electron capture detector to the interfering components. Only experience with applications of the fluorimetric and gas chromatographic methods to the experimental cigarettes can define the relative merits of each procedure.

An analytical liquid chromatograph has been employed in a survey of the possibility of detecting phenanthrene, BaA, and BaP directly in the column effluent. A primary limitation to date is a lack of sufficient sensitivity using an ultraviolet detector. Other detection systems warrant study.

A summary of adsorbents other than alumina evaluated for the separation of the polycyclic hydrocarbons follows:

1. Corasil (Waters Associates)

Column dimensions, 1/4-in o.d. x 100 cm;

Eluents, hexane and hexane half-saturates with water

Results (for separation of mixture of benzene, naphthalene, phenanthrene, BaA, BaP): No detectable resolution.

2. Silica (Woelm, Activity Grade I)

Column dimensions, 1/4-in o.d. x 100 cm;

Eluents, hexane half-saturated with water

Results (above mixture): The three compounds were readily separated from one another, however, higher condensed PAH's such as BaA and BaP are not resolved from one another.

3. Zirconia

Column dimensions, 1/8-in o.d. x 150 cm

Eluent, Hexane

Results (for above mixture): No significant resolution of components in the mixture.

4. XAD-4 (non-ionic resin, Rohm and Haas)

Column dimensions, 1/4-in o.d. x 100 cm

Eluent, Hexane

Results: The retention volumes of the first four components of the above mixture are 18.4, 30.2, 67.0, and 152 ml, respectively. The resin appears to be promising for the separation of PAH's especially with the use of longer columns and a smaller particle size resin (200-300 mesh). One apparent advantage of XAD-4 over alumina should be regeneration of the resin while in the column.

5. XAD-12 (non-ionic resin, Rohm and Haas)

Column dimensions, 1/4-in o.d. x 50 cm

Eluent, Hexane

Results: No detectable resolution

Dimethylsulfoxide (DMSO) is a proven alternative to nitromethane for the preparation of extracts enriched in polycyclic aromatic hydrocarbons. A decided advantage to the use of DMSO is that the aromatic hydrocarbons can reportedly be quantitatively back-extracted into cyclohexane. This eliminates the necessity of evaporating nitromethane and the accompanying difficulty of recovering the polycyclics from the residue.

The results of an experiment in which carbon-14 labelled phenanthrene and benz(a)anthracene were carried through a DMSO extraction follow:

	From MeO ₄ -H ₂ O Into Cyclohexane	From Cyclohexane Into DMSO	From DMSO-H ₂ O Into Cyclohexane
PAH Extracted (%)	101.8	94.99	99.4
No. of Extractions	3	6	2
PAH in Waste (%)	0.24	1.5	----

More than 90% of the activity was recovered in an experiment in which labelled phenanthrene, BaA and BaP were added to condensate and the condensate taken through both the extraction and alumina column procedures. The experiments carried out thus far indicate that a DMSO extraction in our routine procedure could replace the nitromethane extraction.

6.09 INFLUENCE OF CIGARETTE STORAGE CONDITIONS ON CHEMICAL DELIVERY

Cigarettes of known manufacturing date will be available for study over a two-year period. This affords the opportunity to experimentally define those parameters of cigarette storage and handling that may influence the chemical deliveries of the cigarettes. Significant results can only come from a large number of analyses over a long period of time.

Initial studies of the influence of conditioning time and the freeze-thaw operation on the cigarettes are summarized below.

1. Twenty frozen cigarettes were thawed and treated at standard conditions of 60% relative humidity and 75°F. The cigarettes were weighed after conditioning for 3, 23, 25, and 27 hours, and after 9 days. The average weight increase is shown below.

Average weight in mg at:	<u>3 hrs</u>	<u>23 hrs</u>	<u>25 hrs</u>	<u>27 hrs</u>	<u>9 days</u>
	1040	1054	1056.0	1057.5	1060.0

2. Twelve cigarettes were frozen, thawed, conditioned and smoked at standard conditions and were compared to twelve similar cigarettes that were frozen, thawed and conditioned twice before smoking. The following results were obtained.

Average Weight for 12 Cigarettes mg/cig.

<u>Sample No.</u>	<u>Conditions</u>	<u>TPM</u>	<u>Nicotine</u>	<u>Water</u>	<u>Tar</u>
4a	Frozen once	39.16	2.62	4.20	32.30
4b	Frozen twice	41.77	2.74	4.10	35.00
Difference		+2.61	+0.12	-0.10	+2.70
Wt% gain of 4b		+6.8	+4.6	-2.4	+8.4
TPM Wt% gain = $\frac{\text{Wt. in mg of 4b TPM gain} \times 100\%}{\text{Total wt. in mg of 4a TPM}}$					

The cigarettes that were frozen twice showed about 7% increase in TPM and 8% in tar. Water and nicotine was within experimental error.

3. Three sets of 16 cigarettes each were conditioned for different lengths of time and the TPM, water, nicotine, and tar results are listed below.

<u>Sample No.</u>	<u>Conditioning Time</u>	<u>Cig. Wt.</u>	<u>TPM</u>	<u>Water</u>	<u>Nicotine</u>	<u>Tar</u>
IRI-1	40 hrs	1110	40.59	4.17	2.54	33.88
IRI-2	65 hrs	1116	41.60	4.07	2.58	34.95
IRI-3	117 hrs	1112	42.01	4.38	2.52	35.11

A conditioning time of 40 to 117 hours did not show much difference in the chemical composition of the smoke delivered. The differences were about the same as experimental error.

3a. Twelve cigarettes conditioned at 75°F and 60% relative humidity for 28 days were smoked and compared to 12 similar cigarettes conditioned for two days. The results are shown in the following table.

Sample No.	Conditioning Time	Average Wt. in mg per cig.			
		TPM	Nicotine	Water	Tar
8a	2 days	41.26	2.5	4.93	33.83
8b	28 days	36.80	2.23	4.15	30.50
Difference		-4.46	-0.27	-0.78	-3.33
Wt% loss of 8b		10.8	10.8	15.8	9.9
Wt% loss = $\frac{\text{Wt. in mg of 8b TPM loss} \times 100\%}{\text{Total wt. in mg of 8a TPM}}$, calc. were similar for nicotine, etc.					

The cigarettes conditioned for 28 days showed a decrease of about 10% in TPM, nicotine, and tar, and about 16% decrease in water as compared to the cigarettes conditioned for 2 days or normal conditioning.

7.00References

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8.00

TABLE 2 - 1
RESISTANCE TO DRAW
OF
EXPERIMENTAL CIGARETTES OF GIVEN WEIGHT

Cigarette Code	Average R T D (a) (Inches H ₂ O)	Average Weight (a) (Grams)
1	2.6	1.065
2	3.5	1.195
3	3.6	1.211
4	3.7	1.180
5	3.3	1.242
6	3.2	1.179
7	3.8	1.189
8	3.0	1.155
9	2.9	1.170
10	3.2	1.119
11	3.3	1.149
12	4.9	1.085
13	2.3	1.193
14	1.3	1.029
15	2.9	1.194
16	2.8	1.193
17	2.6	1.002
18	2.1	0.972
19	1.7	1.070
20	2.0	1.312
21	>8.0	0.840
22	2.6	1.320
23	2.3	1.259

a) Average of 5 Cigarettes

TABLE 2 - 2
 AVERAGE WEIGHTS AND WEIGHT UNIFORMITY
 FOR GIVEN PACKETS OF THE EXPERIMENTAL CIGARETTES

Cigarette Code	Number of Cigarettes	Average Weight (gm)	Weight Uniformity (gm) ^a	Relative Weight Uniformity ^b
1	30	1.0999	0.0310	-
2	50	1.2164	0.0393	0.50
3	50	1.1795	0.0352	0.56
4	47	1.1983	0.0294	0.67
5	50	1.1969	0.0393	0.50
6	50	1.1776	0.0449	0.44
7	50	1.1907	0.0423	0.47
8	50	1.1929	0.0319	0.62
9	42	1.1984	0.0310	-
10	50	1.1260	0.0287	0.70
11	50	1.1366	0.0347	0.57
12	50	1.0575	0.0259	0.76
13	40	1.2932	0.0588	-
14	50	1.0343	0.0726	0.27
15	50	1.2148	0.0557	0.36
16	40	1.1662	0.0433	-
17	48	1.0449	0.0620	0.32
18	50	1.0491	0.0692	0.29
19	50	1.0638	0.0710	0.28
20	50	1.3011	0.0753	0.26
21	50	0.8178	0.0198	1.00
22	50	1.3818	0.0624	0.32
23	50	1.2352	0.0696	0.28

- a. Standard Deviation; 68% of the cigarettes expected to fall with $\pm$ the value given; larger value = poorer uniformity.
- b. Best Uniformity set equal to 1.00; omitting those values which may be biased by the operators rejection of cigarettes without recording weights.

Table 3-1
Precision of Particulate Matter Analyses
Single 4-Port Analysis Versus Day to Day Variation
Kentucky Reference Cigarette

Standard Deviation

	<u>Puff No.</u>	<u>TPM</u>	<u>Water</u>	<u>Nicotine</u>	<u>Tar</u>
Single Series ^a	0.19	0.69	0.25	0.06	0.46
Day - Day ^b	0.21	1.39	0.45	0.12	1.22

Relative Standard Deviation

	<u>Puff No.</u>	<u>TPM</u>	<u>Water</u>	<u>Nicotine</u>	<u>Tar</u>
Single Series ^a	1.8%	1.6%	4.8%	2.3%	1.2%
Day - Day ^b	1.9%	3.1%	8.3%	4.5%	3.3%

- a. Calculate standard deviation and relative standard deviation for each of 4 sets of 4 pads, average (64 cigarettes).
- b. Calculate standard deviation and relative standard deviation from the direct averaging of all pads run over a three-week period (112 cigarettes).

Table 3-2
Precision of Particulate Matter Analyses
ORNL Versus Literature Values

Average Relative Standard Deviation Within Laboratory

	<u>Puff No.</u>	<u>TPM</u>	<u>Water</u>	<u>Nicotine</u>	<u>Tar</u>
ORNL ^a	1.8%	1.6%	4.8%	2.3%	1.2%
Ogg 12 ^b	2.2	3.5	----	4.2	----
Pillsbury ^c	----	3.7	----	3.7	----
Ogg 8 ^d	2.5	2.6	----	4.6	----

Relative Standard Deviation Between Laboratory

	<u>Puff No.</u>	<u>TPM</u>	<u>Water</u>	<u>Nicotine</u>	<u>Tar</u>
ORNL ^a	1.9%	3.1%	8.3%	4.5%	3.3%
Ogg 12 ^b	6.9	9.8	----	9.6	----
Ogg 8 ^d	1.9	3.2	----	5.2	----
IRI ^e	2.2	6.1	----	9.4	6.1

a. See Table 1

b. Ogg, C. L., J.A.O.A.C. 47, 356-362 (1964); All Colab., 4 ports

c. Pillsbury, H. C., et.al., J.A.O.A.C. 52, 458-462 (1969); less than 100 cigarettes.

d. As b, Best 8 Collab., 4 ports

e. "Proceedings of the Tobacco and Health Conf.," Lexington, Ky., Feb. 24-25, 1970, 23 mm butt.

Table 3-3
Comparison of Inter-Laboratory Agreement

Range of all Results Reported (mg/cig.)

<u>Study</u>	<u>Puff No.</u>	<u>TPM</u>	<u>Water</u>	<u>Nicotine</u>	<u>Tar</u>
ORNL/Collab. ^a	0.68	3.1	0.6	0.15	2.7
Kent. Ref. ^b	0.8	8.0	---	0.82	7.4
Ogg 12 ^c	2.6	11.7	---	0.57	---
Ogg 8 ^d	0.8	2.8	---	0.20	---

a. Average Deviations. See Tables

b. Highest minus lowest value reported in "Proceedings of the Tobacco and Health Conference", Lexington, Kentucky, Feb. 24 and 25, 1970, p.31

c. Highest minus lowest value reported in Ogg, C. L., J.A.O.A.C. 52, 356-362 (1964); all collaborators, 4 ports.

d. As c; best 8 collaborators

Table 3-4
Puffs to reach 23mm Butt

<u>Code</u>	<u>Collaborator</u>	<u>Oak Ridge National Laboratory</u>			
	<u>Best Result (Reported)</u>	<u>Machine Loads (16 Cigs/load)</u>	<u>Best Result (Reported)</u>	<u>Ave. of All Results</u>	<u>Range of All Results</u>
1	-	3	11.2	11.2	0.10
2	11.3	2	10.7	10.7	0.20
3	10.3	2	10.5	10.5	0.05
4	12.2	2	11.7	11.7	0.51
5	10.2	2	9.9	9.9	0.15
6	11.9	2	11.3	11.3	0.11
7	11.1	3	9.9	9.9	0.46
8	11.7	1	12.2	12.2	----
9	8.9	1	9.0	9.0	----
10	12.1	2	10.9	10.9	0.16
11	12.2	2	11.5	11.8	0.62
12	9.7	2	9.1	9.3	0.41
13	----	1	8.9	8.9	----
14	6.1	2	6.0	6.0	0.05
15	9.2	3	7.0	7.4	0.81
16	7.6	1	8.2	8.2	----
17	6.6	1	7.0	7.0	----
18	8.9	3	6.8	7.0	0.41
19	6.8	1	7.0	7.0	----
20	10.9	2	10.2	10.4	0.35
21	5.8	2	6.0	6.1	0.13
22	9.6	2	9.4	9.5	0.10
23	7.6	3	8.8	8.8	0.20

AVERAGE

0.28

Table 3-5
Total Particulate Matter
Milligrams Per Cigarette

Code	Collaborator	Oak Ridge National Laboratory			
	Best Result (Reported)	Machine Loads (16 Cigs/load)	Best Result (Reported)	Ave. of All Results	Range of All Results
1	—	3	44.6	45.4	1.5
2	34.4	2	35.4	35.4	1.9
3	27.8	2	29.5	30.4	1.8
4	42.1	2	44.2	44.2	1.1
5	30.8	2	32.4	32.4	0.9
6	35.5	2	36.4	36.4	0.8
7	30.9	3	31.9	31.8	0.6
8	42.3	1	44.3	44.3	---
9	23.2	1	28.1	28.1	---
10	35.4	2	41.6	41.2	0.7
11	39.1	2	46.1	46.0	0.1
12	31.6	2	33.4	34.1	1.4
13	----	1	16.6	16.6	---
14	14.2	2	16.4	16.4	1.3
15	16.9	3	19.7	20.1	1.1
16	17.5	1	20.0	20.0	---
17	14.7	1	15.9	15.9	---
18	12.7	3	19.2	18.6	1.2
19	16.1	1	20.9	20.9	---
20	34.5	2	37.9	38.5	1.2
21	4.8	2	7.0	7.0	0.1
22	30.4	2	33.4	34.2	1.5
23	28.5	3	35.2	35.7	1.1
AVERAGE					1.1

Table 3-6
Water
Milligrams per Cigarette

<u>Collaborator</u>		<u>Oak Ridge National Laboratory</u>			
<u>Code</u>	<u>Best Result (Reported)</u>	<u>Machine Loads (16 Cigs/load)</u>	<u>Best Result (Reported)</u>	<u>Ave. of All Results</u>	<u>Range of All Results</u>
1	-	3	5.2	5.2	0.5
2	4.2	2	3.7	3.7	0.2
3	3.2	2	2.8	3.1	0.6
4	5.4	2	5.2	5.2	0.2
5	3.8	2	3.6	3.6	0.1
6	4.5	2	4.5	4.5	0.4
7	3.6	3	3.5	3.3	0.6
8	6.0	1	5.8	5.8	---
9	2.5	1	2.9	2.9	---
10	3.5	2	5.3	5.3	0.0
11	4.3	2	5.2	5.4	0.3
12	3.9	2	4.2	4.2	0.0
13	---	1	2.3	2.3	0.0
14	1.3	2	2.9	2.9	1.2
15	2.8	3	3.1	3.0	0.6
16	2.5	1	2.7	2.7	---
17	2.3	1	2.7	2.7	---
18	2.0	3	3.5	2.9	1.2
19	2.7	1	3.7	3.7	---
20	3.9	2	2.9	3.5	1.2
21	1.0	2	0.7	0.7	0.0
22	3.7	2	3.3	3.4	0.3
23	3.5	3	4.2	4.2	0.7
AVERAGE					0.47

Table 3-7
Nicotine
Milligrams per Cigarette

<u>Collaborator</u>		<u>Oak Ridge National Laboratory</u>			
<u>Code</u>	<u>Best Result (Reported)</u>	<u>Machine Loads (16 Cigs/load)</u>	<u>Best Result (Reported)</u>	<u>Ave. of All Results</u>	<u>Range of All Results</u>
1	----	3	2.74	2.77	0.06
2	2.14	2	2.11	2.11	0.13
3	1.82	2	1.94	2.01	0.15
4	2.48	2	2.92	2.92	0.03
5	1.83	2	1.86	1.86	0.00
6	2.38	2	2.26	2.26	0.23
7	1.83	3	2.01	1.98	0.18
8	2.59	1	2.62	2.62	----
9	1.41	1	1.57	1.57	----
10	2.69	2	2.98	2.94	0.09
11	2.69	2	3.09	3.16	0.14
12	3.15	2	3.22	3.35	0.26
13	----	1	0.34	0.34	----
14	0.13	2	0.21	0.21	0.05
15	0.67	3	0.76	0.78	0.16
16	0.89	1	0.92	0.92	----
17	0.68	1	0.76	0.76	----
18	0.62	3	0.82	0.85	0.05
19	0.78	1	0.97	0.97	----
20	1.76	2	2.02	2.10	0.16
21	0.04	2	0.22	0.22	0.01
22	1.31	2	1.47	1.48	0.03
23	1.27	3	1.42	1.49	0.12
AVERAGE					0.11

Table 3-8
Tar
Milligrams per Cigarette

Code	Collaborator	Oak Ridge National Laboratory			
	Best Result (Reported)	Machine Loads (16 Cigs/load)	Best Result (Reported)	Ave. of All Results	Range of All Results
1	----	3	36.6	37.4	1.3
2	28.1	2	29.6	29.6	1.5
3	22.8	2	24.8	25.3	1.0
4	34.2	2	36.2	36.2	0.7
5	25.2	2	27.0	27.0	0.9
6	28.6	2	29.6	29.6	0.3
7	25.5	3	26.4	26.5	0.5
8	33.7	1	35.9	35.9	---
9	19.3	1	23.6	23.6	---
10	29.2	2	33.3	33.0	0.6
11	32.1	2	37.8	37.6	0.5
12	24.6	2	26.0	26.5	1.0
13	----	1	13.9	13.9	---
14	12.8	2	13.4	13.4	0.1
15	13.4	3	15.9	16.3	0.8
16	14.1	1	16.4	16.4	---
17	11.7	1	12.5	12.5	---
18	10.1	3	14.9	14.9	0.5
19	12.6	1	16.3	16.3	---
20	28.8	2	33.0	32.9	0.2
21	3.8	2	6.1	6.2	0.1
22	25.4	2	28.6	29.2	1.3
23	23.7	3	29.7	30.0	0.6
AVERAGE					0.70

Table 3-9
 Comparison of Results - ORNL and Collaborator
 Average Deviation - Puff Number
 Number of Puffs to Reach 23mm Butt

<u>Code</u>	<u>I</u>	<u>II</u>	<u>III</u>
1			
2	-0.6	-0.6	----
3	+0.2	+0.2	0.0
4	-0.5	-0.5	----
5	-0.3	-0.3	----
6	-0.6	-0.6	----
7	-1.2	-1.2	0.0
8	+0.5	+0.5	----
9	+0.1	+0.1	----
10	-1.2	-1.2	
11	-0.7	-0.4	-0.3
12	-0.6	-0.4	-0.2
13	----	-----	----
14	-0.1	-0.1	----
15	-2.2	-1.8	-0.4
16	+0.6	+0.6	----
17	+0.4	+0.4	----
18	-2.1	-1.9	-0.2
19	+0.2	+0.2	----
20	-0.7	-0.5	-0.2
21	+0.2	+0.3	-0.1
22	-0.2	-0.1	-0.1
23	+1.2	+1.2	0.0
Average Deviation	0.68	0.62	0.15

I ORNL Best (reported) Result Minus Collaborator's Result

II ORNL Average Result Minus Collaborator's Report

III ORNL Best (reported) Result Minus ORNL Average Result

Table 3-10
 Comparison of Results - ORNL and Collaborator
 Average Deviation - TPM and Water
 (Milligrams per Cigarette)

Code	TPM			Water		
	I	II	III	I	II	III
1						
2	+1.0	+1.0	----	-0.5	-0.5	----
3	+1.7	+2.6	-0.9	-0.4	-0.4	-0.3
4	+2.1	+2.1	----	-0.2	-0.1	----
5	+1.6	+1.6	----	-0.2	-0.2	----
6	+0.9	+0.9	----	0.0	-0.2	----
7	+1.0	+0.9	+0.1	-0.1	0.0	+0.2
8	+2.0	+2.0	----	-0.2	-0.2	----
9	+4.9	+4.9	----	+0.4	+0.4	----
10	+6.2	+5.8	+0.4	+1.8	+1.8	0.0
11	+7.0	+6.9	+0.1	+0.9	+1.1	-0.2
12	+1.8	+2.5	-0.7	+0.3	+0.3	0.0
13	----	----	----	----	----	----
14	+2.2	+2.2	----	+1.6	+1.6	----
15	+2.8	+3.2	-0.4	+0.3	+0.2	+0.1
16	+2.5	+2.5	----	+0.2	+0.2	----
17	+1.2	+1.2	----	+0.4	+0.4	----
18	+6.5	+5.9	+0.6	+1.5	+0.9	+0.6
19	+4.8	+4.8	----	+1.0	+1.0	----
20	+3.4	+4.0	-0.6	-1.0	-0.4	-0.6
21	+2.8	+2.8	0.0	-0.3	-0.3	0.0
22	+3.0	+3.8	-0.8	-0.4	-0.3	-0.1
23	+6.7	+7.2	-0.5	+0.7	+0.7	0.0
Average						
Deviation	3.1	3.3	0.5	0.6	0.5	0.2

- I ORNL Best (reported) Result minus Collaborator's result
 II ORNL Average Result minus Collaborator's Result
 III ORNL Best (reported) Result minus ORNL Average Result

Table 3-11
 Comparison of Results - ORNL and Collaborator
 Average Deviation - Nicotine and Tar
 (Milligrams per Cigarette)

Code	Nicotine			Tar		
	I	II	III	I	II	III
1						
2	-0.03	-0.03	----	+1.5	+1.5	----
3	+0.12	+0.07	-0.05	+2.0	+2.5	-0.5
4	+0.44	+0.44	----	+2.0	+2.0	----
5	+0.3	+0.3	----	+1.8	+1.8	----
6	-0.12	-0.12	----	+1.0	+1.0	----
7	+0.18	+0.15	+0.03	+0.9	+1.0	-0.1
8	+0.03	+0.03	----	+2.2	+2.2	----
9	+0.16	+0.16	----	+4.3	+4.3	----
10	+0.29	+0.25	+0.04	+4.1	+3.8	+3.3
11	+0.40	+0.47	-0.07	+5.7	+5.5	+0.2
12	+0.07	+0.20	-0.13	+1.4	+1.9	-0.5
13	----	----	----	----	----	----
14	+0.08	+0.08	----	+0.6	+0.6	----
15	+0.09	+0.11	-0.02	+2.5	+2.9	-0.4
16	+0.03	+0.03	----	+2.3	+2.3	----
17	+0.08	+0.08	----	+0.8	+0.8	----
18	+0.20	+0.23	-0.03	+4.8	+4.8	0.0
19	+0.19	+0.19	----	+3.7	+3.7	----
20	+0.26	+0.34	-0.08	+4.2	+4.1	+0.1
21	+0.18	+0.18	0.0	+2.3	+2.4	-0.1
22	+0.16	+0.17	-0.01	+3.2	+3.8	-0.8
23	+0.15	+0.22	-0.07	+6.0	+6.3	-0.3
Average						
Deviation	0.15	0.17	0.05	2.7	2.8	0.6

I ORNL Best (reported) Result minus Collaborator's Result

II ORNL Average Result minus Collaborator's Result

III ORNL Best (reported) Result minus ORNL Average Result

Table 3-12
Ranking of Cigarettes According to Average Deliveries
Nicotine and Tar

		Cigarette Code Number					
		Nicotine			Tar		
		mg/Cig.	mg/g Tob.	Wt.% TPM	mg/Cig.	mg/g Tob.	Wt.% TPM
Highest Delivery		12	12	12	11	11	20
		11	11	10	1	1	21
		10	10	11	4	4	22
		4	1	4	8	10	23
		1	4	3	10	8	3
		8	8	7	20	6	9
		6	6	6	23	2	13
		2	2	1	6	20	2
		20	7	2	2	12	5
		7	3	8	22	23	7
		3	5	5	5	5	1
		5	20	9	7	7	11
		9	9	20	12	22	4
		22	23	17	3	3	16
		23	22	19	9	9	6
		19	19	16	16	18	14
		16	18	22	19	19	8
		18	16	18	15	16	15
		17	17	23	18	14	10
		15	15	15	13	15	17
Lowest Delivery		13	21	21	14	17	12
		21	13	13	17	13	19
		14	14	14	21	21	18

Table 3-13
 Ranking of Cigarettes According to Average Deliveries
 TPM and Water
 Cigarette Code Number

	TPM		Water		
	<u>mg/Cig.</u>	<u>mg/g Tob.</u>	<u>mg/cig.</u>	<u>mg/g Tob.</u>	<u>Wt.% TPM</u>
Highest	11	11	8	1	18
Delivery	1	1	10	8	14
	8	10	1	10	19
	4	4	4	11	17
	10	8	11	4	15
	20	12	6	12	13
	6	6	12	18	16
	2	2	23	19	8
	23	20	2	23	10
	12	23	19	2	12
	22	5	5	6	6
	5	7	7	14	4
	7	22	18	5	23
	3	3	22	7	1
	9	9	15	15	11
	19	18	9	17	7
	16	19	14	9	5
	15	16	20	16	2
	18	14	3	22	9
	13	15	16	3	21
	14	17	17	20	22
Lowest	17	13	13	13	3
Delivery	21	21	21	21	20

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5

5

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J. Comput. Anal. **6**(1987), no. 1, 1–10. MR88m:65041 (88j:65001)

(5)

Lowest
Delivery

Table 3-16

Ranking of Cigarettes According to Nicotine Delivery
in Milligrams per Cigarette
ORNL and Collaborator
Cigarette Code Number

	<u>ORNL</u>	<u>Collaborator</u>
Highest	12	12
Delivery	11 } - Same Within $\pm \sigma$	10 } - Same
	10 } - Same Within $\pm \sigma$	11 }
	4 }	8
	8	4
	6	6
	2 }	2
	20 } - Same Within $\pm \sigma$	5 }
	7 }	7 } - Same
	3	3
	5	20
	9	9
	22	22
	23	23
	19 } - Same Within $\pm \sigma$	16
	16 }	19
	18	17
	17 } - Same Within $\pm \sigma$	15
	15 }	18
	21 } - Same Within $\pm \sigma$	14
	14 }	21
Lowest		
Delivery		

Table 3-17

Summarized Results

Reporting Date: 8-24-70

Date Analyzed: July - August 1970

Last Report: 6-29-70

Samples: Experimental Cigarettes, # 1-23

Units: Milligrams per Puff

Sample	TPM	Water	Nicotine	Tar
1	3.98	0.46	0.25	3.27
2	3.30	0.35	0.20	2.77
3	2.81	0.27	0.18	2.36
4	3.78	0.44	0.25	3.09
5	3.27	0.36	0.19	2.73
6	3.22	0.40	0.20	2.62
7	3.22	0.35	0.20	2.67
8	3.63	0.48	0.21	2.94
9	3.12	0.32	0.17	2.62
10	3.82	0.49	0.27	3.06
11	4.01	0.45	0.27	3.29
12	3.67	0.46	0.35	2.86
13	1.87	0.26	0.04	1.56
14	2.73	0.48	0.04	2.23
15	2.81	0.44	0.11	2.27
16	2.44	0.33	0.11	2.00
17	2.27	0.38	0.11	1.78
18	2.82	0.51	0.12	2.19
19	2.99	0.53	0.14	2.33
20	3.72	0.28	0.20	3.24
21	1.17	0.55	0.04	1.02
22	3.55	0.35	0.16	3.04
23	4.00	0.48	0.16	3.38

Table 3-18

Ranking of Cigarettes According to average Deliveries

Milligrams per Puff

Cigarette Code Number

	<u>TPM</u>	<u>Water</u>	<u>Nicotine</u>	<u>Tar</u>
Highest	11	21	12	23
Delivery	23	19	10	11
	1	18	11	1
	10	10	1	20
	4	8	4	4
	20	14	8	10
	12	23	2	22
	8	1	6	8
	22	12	7	12
	2	11	20	2
	5	4	5	5
	6	15	3	7
	7	6	9	6
	9	17	22	9
	19	5	23	3
	18	2	19	19
	3	7	18	15
	15	22	15	14
	14	16	16	18
	16	9	17	16
	17	20	13	17
		3	14	13
Lowest	13		21	21
Delivery	21	13		

Table 3-19

Influence of Minor Experimental Variation on Analysis of Particulate Matter
Kentucky Reference Cigarette

<u>Run No.</u> <u>Date Opened</u>	<u>Date</u> <u>Smoked</u>	<u>Draft</u> <u>cfm</u>	<u>Ave. Wt.</u> <u>(gm.)</u>	<u>No.</u> <u>Cigs.</u>	<u>Puffs</u>	<u>TPM</u>	<u>Water</u>	<u>Nicotine</u>	<u>Tar</u>
1(7-20)	7-22	50	1.108	16	11.2	44.4	5.3	2.64	36.5
2(7-20)	7-22	80	1.110	16	10.9	43.7	5.4	2.63	35.7
3(7-20)	7-22	80	1.109	16	11.1	42.9	5.3	2.51	35.1
4(7-20)	7-23	0	1.086	16	11.2	46.1	5.5	2.72	37.9
5(7-20)	8-3	50	1.102	16	11.4	45.6	5.3	2.80	37.5
6(7-20)	8-4	50	1.102	4	11.3	44.8	5.9	2.61	36.3
7(8-2)	8-4	50	1.128	4	11.1	45.8	6.4	2.74	36.7
8(8-2)	8-5	50	1.102	4	11.2	46.4	5.8	2.56	38.0
9(8-2)	8-6	50	1.080	4	11.0	43.9	6.0	2.85	35.0
10(8-8)	8-10	50	1.115	16	11.3	45.5	4.9	2.80	37.8
11(8-8)	8-11	50	1.104	16	11.2	44.6	5.2	2.74	36.6
<u>Average</u>			1.104		11.2	44.9	5.5	2.69	36.6

Table 3-20

Influence of Minor Experimental Variation on Analysis of Particulate Matter

Code 2

Run No. Date Opened	Date Smoked	Draft cfm	Ave. Wt. (gm.)	No. Cigs.	Puffs	TPM	Water	Nicotine	Tar
1(7-20)	7-23	50	1.190	16	10.2	33.2	3.4	2.04	27.8
2(7-20)	7-23	0	1.197	16	10.8	36.4	3.8	2.16	30.4
3(7-20)	8-4	50	1.200	4	10.3	34.1	4.2	1.87	28.0
4(8-2)	8-4	50	1.251	4	10.8	35.6	3.6	2.15	29.9
5(7-20)	8-5	50	1.198	4	10.9	36.7	4.4	2.05	30.2
6(8-2)	8-5	50	1.242	4	11.0	36.4	4.0	----	----
7(8-2)	8-6	50	1.201	4	10.4	32.8	4.2	1.89	26.7
8(8-8)	8-10	50	1.217	16	10.6	34.5	3.6	2.04	28.9
Average			1.212		10.6	35.0	3.9	2.03	28.8

Table 3-21

Agreement Between ORNL and Collaborator

High and Low Fume Hood Velocity

Average Absolute Difference

<u>Fume Hood</u>	<u>Puff No.</u>	<u>TPM</u>	<u>Milligrams per Cigarette</u>		
			<u>Water</u>	<u>Nicotine</u>	<u>Tar</u>
High Velocity	1.5	1.9	0.9	0.16	1.7
Low Velocity	0.7	3.1	0.6	0.15	2.7

Table 4-1

Typical Formaldehyde Results Using a Dynamic Trapping System

<u>Port No.</u>	<u>Micrograms Formaldehyde per Cigarette</u>	
	<u>Commercial Brand</u>	<u>Kent Ref. Cig.</u>
1	45.1	25.6
2	46.5	23.8
3	45.7	24.1
4	44.0	23.4
<hr/>	<hr/>	<hr/>
Average	45.3	24.2

Table 4-2

Oxides of Nitrogen in a Commercial Cigarette

As Determined By Non-Aqueous Titration

Nitrogen Dioxide (ug/cig.)

Port No.	Potentiometric Endpoint	Visual Endpoint	Potentiometric Endpoint With Acid Spike
1	214	201	182
2	232	203	195
3	230	217	199
4	248	---	182
—	—	—	—
Average	231	207	190

Table 4-3

Hydrogen Cyanide Delivery of the Kentucky Reference Cigarette

Hydrogen Cyanide
(Micrograms per Cigarette)

Port No. ^a	Gas Phase		Whole Smoke		
	Method of Schilt	Ascarite Method		Ascarite Method	Reported Average ^b
1	214	235	243	345	---
2	240	229	---	375	---
3	260	208	200	445	---
4	240	235	219	---	---
Average	238	227	221	388	418

a. Each series of values correspond to a different sampling of the Kentucky Reference Cigarette.

b. "Proceedings of the Tobacco and Health Conference", Lexington, Kentucky, Feb 24 and 25, 1970 (pg 31).

Table 6-1

Polarographic Behavior of N-nitrosamines

Compound	Mol. Wt.	E1/2 vs SCE	Notes
N-nitrosodimethylamine	74.1	-0.880	
N-nitrosodiethylamine	102.1	-0.834	
N-nitrosopiperdine	114.1	-0.734	Small second wave at -0.9v
N-nitrosopropylamine	130.2	-0.792	
N-nitrosodiphenylamine	198.2	-0.510	
9-nitrosocarbazole		No Reduction Wave	

Table 6-2

Gas Chromatographed Cigarette Smoke - Related Phenols

<u>Number</u>	<u>Name</u>	<u>Number</u>	<u>Name</u>
1	Phenol	16	m-Methoxyphenol
2	o-Cresol	17	2,6-Dimethoxyphenol
3	m-Cresol	18	2,4,6-Trimethylphenol
4	p-Cresol	19	p-Methoxyphenol
5	Guaiacol	20	Thymol
6	Salicylaldehyde	21	γ -Naphthol
7	o-Hydroxyacetophenone	22	2,4-Xylenol
8	2,6-Xylenol	23	4-Ethylphenol
9	2,3-Xylenol	24	Eugenol
10	2,3,5-Trimethylphenol	25	Isoeugenol
11	3-Ethylphenol	26	Coumarin
12	2-Ethylphenol	27	Vanillin
13	3,4-Xylenol	28	β -Naphthol
14	3,5-Xylenol	29	Methyl-p-hydroxybenzoate
15	2,5-Xylenol	30	o-Phenylphenol

Figure 2-1. Four-port version of the Phillip Morris Automated Smoking Machine - Smoking Machine No. 1.

Figure 2-2. ORNL-constructed four-port smoking machine for the precise collection of samples of the gas phase of smokes. Shown interfaced to the cryothermal gas chromatograph. Smoking Machine No. 2.

ORNL-DWG. 70-5396

Figure 2-3. Flow diagram of Smoking Machine No. 2.

Figure 2-4. Modified Filamatic Vial-Filler Smoking Machine. Shown interfaced to a gas phase pH assembly. Smoking Machine No. 3.

Figure 2-5. Smoking Machine for the Production of Cigarette Smoke Condensate. Smoking Machine No. 4.

ORNL-DWG. 70-7752

Figure 2-6. Filter Assembly Leak Test Apparatus.

Figure 2-7. Flow Diagram of ORNL Resistance-to-Draw Apparatus.

Figure 2-8. ORNL Resistance-to-Draw Apparatus.

Figure 2-9. Griffith Steam Distillation Apparatus.

ORNL-DWG 70-10788

Figure 3-1. Gas chromatographic separations of 1. water 2. acetone 3. isopropanol 4. n-propanol 5. ethanol and 6. p-dioxane on porous polymer columns.

Figure 3-2. Chromatograms generated in the determination of water in total particulate matter. Ten microliter injections of standards containing 5, 10, 15 mg water in 20 ml 99.7-0.3 p-dioxane-isopropanol 1. air 2. water 3. isopropanol 4. p-dioxane.

ORNL-DWG 70-10786

Figure 4-1. Valve interface between Smoking Machine No. 2 and cryothermal gas chromatograph.

Figure 4-2. Gas chromatogram generated by one cigarette in the analysis of the gas phase for acetaldehyde and acrolein content. Original column.

Figure 4-3. Gas chromatogram generated by one cigarette in the analysis of the gas phase for acetaldehyde and acrolein. New column.

ORNL-DWG 70-10785

Figure 4-4. Trap configuration for the dynamic collection of formaldehyde in the gas phase of smokes.

Figure 4-5. Dynamic gas phase traps installed on the rear of the automated smoking machine.

Figure 5-1. Gas chromatographic separation of 1. methanol 2. nicotine 3. dimethylphthalate and 4. nornicotine.

Figure 5-2. Gas chromatographic separation of 1. phenol 2. o-Cresol 3. m-Cresol plus p-Cresol and 4. ether.

Figure 6-1. Gas chromatographic component profile of smoke condensate from the Kentucky reference cigarette. 3% OV-101 on 80-100 mesh AW-DMCS chromosorb G in 20-ft. of 1/8-in. stainless steel, $70^{\circ} \xrightarrow[6 \text{ min}]{}$ $70^{\circ} \xrightarrow[5^{\circ}/\text{min}]{}$ 250, 10 ml/min N_2 .

Figure 6-2. Sulfur-specific gas chromatograms from replicate samples of the gas phase of a non-filtered cigarette. 1. Unknown 2. Hydrogen sulfide 3. Carbonyl sulfide 4. Unknown. 6-ft. x 1/4-in. glass, 80-100 mesh Porapak Q, 50°C, 80 ml/min N₂.

ORNL - DWG. 70-8609

Figure 6-3. Deliveries of sulfur compounds - gas phases of filter (F) and nonfilter (NF) cigarettes. 6-ft. x 1/4-in glass, 80-100 mesh Porapak Q, 50°C, 80 ml/min N₂.

ORNL-DWG. 70-8610

Figure 6-4 Deliveries of sulfur compounds - gas phase of nonfilter(NF), charcoal filter (CF), and charcoal filter cigarettes with filters removed (FR). 6-ft. x 1/4-in glass, 80-100 mesh Porapak Q, 50°C, 80 ml/min N₂.

Figure 6-5. Sulfur-specific programmed temperature chromatograms - gas phases of non-filter and charcoal filter cigarettes. 6-ft. x 1/4-in. glass, 80-100 mesh chromosorb 104, 80 ml/min N₂, 50° 5°/min 150°C.

Figure 6-6. Gas chromatographic separation of monohydric phenols reported present in cigarette smoke. See Table 6-2.

Figure 6-7. Gas chromatographic separation of monohydric phenols reported present in cigarette smoke. See Table 6-2.

Figure 6-8. Gas chromatographic separation of monohydric phenols reported present in cigarette smoke. See Table 6-2.

Figure 6-9. Gas chromatographic separation of monohydric phenols reported present in cigarette smoke. See Table 6-2.

Figure 6-10. Gas chromatographic separation of nitrogen compounds reported present in cigarette smoke.

9.00 APPENDIX A PRELIMINARY ANALYTICAL PROCEDURES

The procedures which follow are intended not to be definitive, but only descriptive. Each procedure is subject to substantial modification, particularly as regards details of the apparatus and operations involved. The status of each procedure can be determined by a consideration of previous sections of this report.

It is anticipated that a compilation of formally prepared, precisely defined analytical procedures will be one product of this activity. Such procedures should include descriptions of the reliability of the method, and of the likely sources of error. As such, these procedures can only be prepared after substantial experience with its application, and a study of the results it provides. These formal procedures will be prepared as each attains this stage of maturity.

PRELIMINARY PROCEDURES

<u>PAGE</u>	<u>SEC.</u>	<u>PROCEDURE</u>
114	9.01	Total Particulate Matter of Cigarette Smoke
118	9.02	Nicotine Alkaloids in Total Particulate Matter
121	9.03	Water in Total Particulate Matter
123	9.04	Tar Content of Total Particulate Matter
124	9.05	Acetaldehyde and Acrolein in the Gas Phase
126	9.06	Carbon Monoxide and Carbon Dioxide in the Gas Phase
128	9.07	Oxides of Nitrogen in the Gas Phase
131	9.08	Formaldehyde in the Gas Phase
134	9.09	Hydrogen Cyanide in the Gas Phase
137	9.10	pH of the Gas Phase
139	9.11	Water and Acetone in Smoke Condensate Preparation
141	9.12	Nicotine and Nornicotine in Smoke Condensate
143	9.13	Nicotine Alkaloids in Smoke Condensate
145	9.14	Phenol, o-Cresol and m-Cresol plus p-Cresol in Smoke Condensate
147	9.15	Phenol Number of Smoke Condensate

<u>PAGE</u>	<u>SEC.</u>	<u>PROCEDURE</u>
150	9.16	Phenanthrene, Benz(a)anthracene, and Benzo(a)pyrene in Smoke Condensate
157	9.17	Weak and Very Weak Acids in Smoke Condensate
159	9.18	pH of Smoke Condensate
161	9.19	Operation of the Phipps and Bird Four-Port Smoker
166	9.20	Operation of the Griffith Still

Procedure PM-1 (Preliminary)

Total Particulate Matter of Cigarette Smoke

Apparatus

All the apparatus listed in items 1 through 6 are described by Ogg⁽¹⁾, and can be obtained from Phipps and Bird Inc., Richmond, Va.

1. Cigarette smoking machine, 4-port, constant volume Phipps and Bird.
2. Filter holder, threaded lucite inner and outer parts with a neoprene gasket.
3. Filter disc, 44 mm in diameter, CM113A. The fiberglass filter must collect at least 99% of all particles 0.3 microns in diameter and over, and at a flow rate of 28 linear ft. per min, have a pressure drop not exceeding 93 mm of water at 28-ft. per min, and contain not more than 5% acrylic fiber.
4. Lucite guide to help position the rubber o-ring and rubber membrane on the filter holder.
5. Rubber membrane about 3.5 cm x 3.5 cm square cut from medium dental dam sheeting. The sheet is placed on a solid rubber stopper about 3-in in diameter and a hole 4 to 6 mm in diameter is punched in the center of the sheet with a sharp cork borer of appropriate size.
6. Rubber o-rings, neoprene, 3/8-in inside diameter.
7. Vials, caps, and Teflon lined rubber diaphragms of 28 ml size, supplied by Schwarz Bio Research, Mountain View Ave., Orangeburg, New York.
8. Water manometer, about 600 mm high for leak tests on Cambridge filters. This can be made by bending about 7-ft. of 8 mm glass tubing in an "S" shape with "U" bends and attaching rubber hose to an appropriate vacuum.

Procedure

1. From the supply of frozen cigarettes, in sealed polyethylene bags of 50 cigarettes per bag, remove one bag of cigarettes, and

allow them to thaw at room temperature for about 8 hours, then open the bag and place the cigarettes in wire baskets. Condition the cigarettes in a room at $75^{\circ} \pm 2^{\circ}$ F and $60\% \pm 2\%$ relative humidity for at least 30 hours.

2. Examine each of the cigarettes and remove all cigarettes that have obvious bad qualities; such as, tobacco loss, torn wrappers, and smashed ends, etc.
3. Weigh the remaining cigarettes to the nearest milligram and record their weights.
4. Select 4 sets of cigarettes of comparative weights all of which are within ± 20 mg for the smoking machine.
5. Prepare the filter holder.
 - a. Place a conditioned Cambridge filter in the female half of the filter holder with the soft absorbant side of the filter facing the opening for the cigarette.
 - b. Place a neoprene o-ring, 44 mm outside diameter against the filter and assemple the male half of the filter holder.
 - c. Leak test the assembled filter holder. The filter should hold a head of water 380 to 400 mm high on a water manometer.
 - d. Place a $3/8$ -in neoprene o-ring on the tapered end of the lucite guide.
 - e. Place a rubber membrane of the off-set end of the guide, then place the off-set end of the guide against the largest opening in the filter holder and roll the o-ring against and over the rubber membrane and onto the projection of the filter holder.
 - f. Weigh the completely assembled filter holder on an analytical balance to the nearest 0.1 mg.
 - g. Place the small projection of filter holder No. 1 in the rubber tube coupling at port No. 1 on the cigarette machine.
 - h. Assemble, leak check, and mount all four filter assemblies in a similar way.
6. Pull the thread taut across the micro switches and secure it by sliding it through the spring coils.

7. Place the first cigarette from cigarette set No. 1 in the rubber membrane at port No. 1, and adjust the cigarette to a 23 mm butt length with the micro switch release thread resting on the 23 mm butt mark. Mount all four cigarettes in like manner.
8. Adjust the cigarette lighters to light all four cigarettes at the same time. The lighters should be pre-heated for about 30 sec in order to light the cigarettes well during the first 2 sec puff.
9. Carefully observe the smoking of the cigarettes, correct any leaks or non-uniform smoking conditions and re-run any cigarettes smoked under obvious leaking or improper conditions.
10. After the smoking cigarette burns through the thread that releases the micro switch, allow the cigarette butt to remain in the holder about 50 sec to avoid losing the smoke from the last puff.
11. Remove the cigarette butts, record the electronic timer counts, and number of puffs required to smoke each cigarette.
12. Smoke 4 cigarettes on each filter, using one filter at each port.
13. After the last cigarette is smoked, immediately remove the complete filter holder assembly and re-weigh to the nearest 0.1 of a milligram.

Total Particulate Matter Treatment for Water and Nicotine Determinations

1. Immediately after weighing the filter holder assembly, carefully remove the filter and place it in a pre-dried 28 ml vial and cap it with a Teflon lined rubber diaphragm. Wipe out the smoke condensate on the inside of the filter holder with one-fourth of a filter and add it to the vial.
2. Add 20.0 ml of dioxane solution ^(a) as soon as possible. The vial should be kept tightly closed except when additions are made and all additions should be made as quickly as possible to prevent loss of sample and water pick-up from the atmosphere.
3. Allow the sample to set for at least 12 hours before analyzing for water followed by nicotine determination.

Calculations

1. Total particulate matter including water.

$$\text{TPM in mg per cigarette} = \frac{A-B}{4} \times 1000$$

Where A = weight in grams of filter holder assembly before smoking.

B = weight in grams of filter holder assembly after smoking four cigarettes.

References

1. Off, C. L., J.A.O.A.C. 47, (No. 2) pp. 356-362, 1964.

(a). The dioxane solution is 0.3% isopropanol. It is made by adding 3.0 ml of isopropanol to a one liter flask and filling the flask to volume with dry dioxane.

Procedure PM-2 (Preliminary)

The Steam Distillation and Spectrophotometric Determination of
Total Alkaloids as Nicotine
in the Total Particulate Matter of Cigarette Smoke

Apparatus

1. Cigarette smoking machine, Phipps and Bird, Inc., Catalog No. 9-90 or equivalent.
2. Filter holder, as described by Ogg⁽¹⁾, consisting of threaded inner and outer parts and neoprene gasket.
3. Filter disc, CM113A glass fiber, 44 mm diameter from Cambridge Filter Corp., East Syracuse, New York.
4. Rubber membrane, dental dam, 3.5 x 3.5 cm, with 4-6 mm hole in center.
5. Rubber o-ring, 3/8-in inside diameter.
6. Vials, 28 ml volume with caps and 22 mm Teflon-lined rubber diaphragms, Catalog No 85-0003 from Swartz Bio Research, Inc., Orangeburg, New York 10962.
7. Griffith steam distillation apparatus, 6-port unit from Lab Glass, Inc., Kingsport, Tennessee.
8. Six - 250 ml short form, wide mouth, flat bottom volumetric flasks.
9. Spectrophotometer, Cary Model 14, Beckman DU, or equivalent.
10. Magnetic stirring motor and Teflon stirring bar (1-in x 1/2-in).

Reagents

1. p-Dioxane, spectroquality grade from Matheson, Coleman and Bell, East Rutherford, New Jersey.
2. 2-Propanol, "Baker Analyzed" reagent (9084) from J. T. Baker Chemical Company, Phillipsburg, New Jersey.
3. Hydrochloric acid, ~ 36% reagent grade for preparation of 0.2 M HCl and 1 + 9 (HCl + H₂O) solution.
4. Sodium hydroxide-salt solution. Dissolve 300 grams NaOH in distilled water and dilute to 1 liter. Saturate with NaCl, and

cool. Stir and after 24 hours decant the supernate.

5. Nicotine, purified by vacuum distillation. Prepare a standard solution by weighing 1 gram and diluting with 0.05 N HCl to a 100 ml volume. Prepare fresh each 30 days.

Procedure

Four sets of four cigarettes each are smoked through standard glass fiber filters. The cigarettes are smoked to precisely 23 mm butt lengths, using 2 sec puffs of 35 cc volume taken once per minute in a room maintained at 75°F and 60% relative humidity. The weight increase of each filter pad attending the smoking of the four cigarettes is determined, recorded as the total particulate matter delivered by the cigarettes, and the filter pads are immersed in p-dioxane in sealed containers. The water contents of the p-dioxane extracts are determined and the extracts are then analyzed for nicotine content.

Four sealed vials, containing a smoked filter pad, one-quarter of an unsmoked pad used to wipe the filter assembly, and 20 ml of p-dioxane are subjected to analysis.

Analysis of the p-dioxane Extracts of Total Particulate Matter

1. Prepare the Griffith still for operation.
2. Pipette a 5 ml aliquot of 0.2 H hydrochloric acid into each of the four still pots.
3. Pipette a 10 ml aliquot of the dioxane extract into each of the still pots. Pipette no more than a 1.0 ml aliquot of a 10 µg/ml nicotine standard into a still pot that contains 10 ml of dioxane and 5 ml 0.2 H hydrochloric acid solution.
4. Steam distil each acid solution until approximately 100 ml of distillate is collected.
5. Discard this distillate, and place a short form, wide mouth 250 ml volumetric flask containing 10 ml of 1:9 HCl:H₂O under each U tube receiver.
6. Add 5 ml of the sodium hydroxide-saturated sodium chloride solution to each still pot and steam distil until approximately 230 ml of distillate is collected in each flask.
7. Dilute each distillate to 260 ml with distilled water.

8. Measure the absorbance of each distillate at three wave lengths: 236, 259, and 282 mμ, respectively with a spectrophotometer using a 0.05 M hydrochloric acid solution as the reference blank.

Calculations

The concentration of nicotine in each distillate is calculated in the following manner:

$$1. A'_{259} = 1.059 \times [A_{259} - 1/2 (A_{236} + A_{282})]$$

Where A = the absorbance reading at the subscripted wave length
and A'_{259} = Reading corrected for background absorbance

$$2. \frac{A'_{259}}{a} \times V = \text{mgs of nicotine in distillate}$$

Where V = Volume of distillate times dilution factor (if any).

a = $A/(c \times b)$ (accepted value = 34.3)

A = Absorbance reading of a standard nicotine solution at 259 mμ

c = Concentration of nicotine in a 0.05 N HCl solution in g/l

b = Cell length.

$$3. \text{mgs nicotine in distillate} / 2 = \text{mgs nicotine per cigarette.}$$

References

1. Ogg, C. L., J.A.O.A.C. 47, 356 (1964).
2. Pillsbury, H. C., et al, J.A.O.A.C. 52, 458-462 (1969).
3. Schultz, F. J. and Spears, A. W., Tobacco Sci. 10, 75 (1966).
4. Griffith, R. B., Tobacco Sci. 1, 130-137 (1957).

Procedure PM-3 (Preliminary)

Determination of Water in Particulate Matter

Apparatus

1. Gas chromatograph, MicroTek No. 160. Column: 4-ft. x 0.25-in copper packed with 80-100 mesh Porapak Q. Column temperature = 190°C, Inlet temperature = 200°C, Detector temperature = 210°C, Helium carrier gas flow rate $\cong$ 80 ml/min, Thermal conductivity detector current = 150 ma, Attenuation adusted to give reasonable internal standard peak.
2. Thirty-five milliliter capacity screw-topped vials with Teflon-faced diaphragm caps or rubber septum-stoppers.
3. Twenty-five microliter capacity precision syringe.
4. Vortex mixer.

Reagents

1. 99.7% - 0.3% dry dioxane-isopropanol.

Procedure

One vial containing the smoked filter pad, and a quarter-pad used to wipe the filter holder, plus 20 ml of dioxane-isopropanol will be available from each port smoked. A vial containing 20 ml of dioxane-isopropanol in contact with one and one-quarter unsmoked filter pads serves as the water blank.

Analysis of Sample Extracts

1. Mix the particulate matter with a vortex mixer, taking care to avoid disintegrating the filter pad.
2. Inject at least three 10 μ l aliquots of the extracts from each port directly into the gas chromatograph. Similarly, inject 10 μ l aliquots of the water blank.
3. Note and measure the heights of the peaks corresponding to water and isopropanol. Determine the ratio of the peak heights of water to isopropanol. Average the ratios obtained from multiple injects of a given extract. Subtract the ratio determined for the blank from those corresponding to each port. Compare these blank-corrected

ratios to a calibration curve to determine the total water content of each filter pad.

Preparation of a Calibration Curve

1. Draw 20 ml of the extracting solvent into each of five vials of the type used for extracting the sample. Add 5.0 μ l, 10.0 μ l, 15.0 μ l, and 20.0 μ l of water to four of the vials. Reserve the fifth vial as a standard blank.
2. Treat these standards in the same way the samples are treated. Construct a calibration curve by plotting the water content (in milligrams) of each vial versus the blank-corrected peak height ratio H_2O :isopropanol.

Reference

1. Schultz, F. J., and Spears, A. W., Tob. Sci. 10, 75 (1966)

Procedure PM-4 (Preliminary)

Determination of "Tar" in Particulate Matter

"Tar" is defined as the weight of the total particulate matter minus the combined weights of the water and the alkaloids expressed as nicotine contents of the particulate matter. If the value determined for total particulate matter, water, or nicotine alkaloids for any given port is considered suspect, a value is not computed for "tar" for that port.

Reference

1. Ogg, C.L., J.A.O.A.C. 47, 356 (1964).

Procedure GP-1

Acetaldehyde and Acrolein in Cigarette Smoke
Cryothermal Gas Chromatographic Method

Apparatus

1. Cigarette smoking machine number 2.
2. Gas chromatograph. The MT-150, with the CT-75 cryothermal system, Tracor, Inc., Austin, Texas.
3. 50-liter liquid nitrogen container.
4. Gas chromatographic column, 4-ft. x 1/4-in. Burrell Ucon in series with a 4-ft. x 1/4-in. 20% β - β' oxydipropionitrile column.
5. Gas chromatographic detector, H_2 flame ionization.
6. Cigarette holder, plastic.
7. Rubber membrane, dental dam, 3.5 x 3.5 cm, with 4-6 mm hole in center.
8. Rubber o-ring, 3/8-in. inside diameter.

Procedure

1. Preparation of sample. Condition the cigarettes at $75 \pm 2^\circ C$ and $60 \pm 2\%$ relative humidity for 24 hours or until they are to be smoked. Determine the average cigarette weight. Select three good cigarettes in the weight range ± 20 mg from the average weight. Reject cigarettes with soft spots or loose ends. Mark each cigarette at the butt length to which it is to be smoked, taking care not to puncture the cigarette paper.
2. Adjustment of smoking machine. Adjust the smoking machine to take a 35 ± 0.5 ml puff of 2 ± 0.2 sec duration every 60 ± 1 sec. The machine should be checked daily and adjusted as necessary.
3. Smoking cigarettes. Use a fresh Cambridge filter for each cigarette.
 - A. Place cigarette in cigarette holder. Set mode selector of temperature programmer to Iso. Set mode selector of CT-75

to Full Cool. Set Initial Temp. dial to 125⁽¹⁾, and Final Temp. dial to 215⁽¹⁾.

- B. Set Initial Hold timer to 10, program rate selector to 25°/min, Final Hold timer to ∞, Cool timer to 0, Input Attenuator to 10³, and Output Attenuator to 16.
- C. When the column oven temperature reaches -50°C⁽²⁾, switch CT-75 mode selector to Sub-Ambient.
- D. When the temperature reaches -75°C, turn on smoking machine. Check time of solenoid actuation by sweep second hand on clock.
- E. Have a lighted match ready ~ 2 sec in advance of solenoid actuation. Light cigarette on puff.
- F. Smoke to 23 mm butt length; count the number of puffs. The GC is automatic until 30 min after cigarette is lit.
- G. At exactly 30 min after lighting the cigarette, adjust final hold temperature to 35°C and set output attenuator to 4.
- H. After acrolein is eluted, reset mode selector on programmer to +75°C, and set mode selector on CT-75 to normal.
- I. Continue chromatogram until no more peaks are eluted (~ 15 min).
- J. CT-75 control can be reset immediately as in Step "A" to begin the next run. However, liquid nitrogen will be saved if the column is air-cooled to room temperature before starting the cryothermal cycle. Smoke three cigarettes from each sample batch by repeating Steps "A" through "J".

Notes

1. The CT-75 cryothermal programmer is designed to have a 200° off-set in temperature settings to allow the use of positive reading dials for below ambient temperature. Dial settings must be the temperature desired +200°; therefore, 125° initial temperature is actually -75°C, whereas, 215° final hold temperature is actually +15°C.
2. The Sub-ambient setting on the CT-75 mode selector actuates a pulsing relay that gives precise control of liquid nitrogen to column oven.

Procedure GP-2 (Preliminary)

Carbon Monoxide and Carbon Dioxide
in Cigarette Smoke
Gas Chromatographic Method

Apparatus

1. Cigarette smoking machine number 1.
2. Adapter from smoking machine outlet to a rubber balloon.
3. Rubber balloons.
4. 1 ml gas-tight syringe.
5. Gas chromatograph. The MT-150, Tracor, Inc., Austin, Texas.
6. Gas chromatographic columns.
 - a. CO-molecular sieve 5A, 3-ft. x 1/4-in., 30-60 mesh.
 - b. CO₂-Porapak Q, 6-ft. x 1/4-in., 50-80 mesh.
7. Gas chromatographic detector, thermal conductivity.
8. Cigarette holder, plastic.
9. Rubber membrane, dental dam, 3.5 x 3.5 cm, with 4-6 mm hole in center.
10. Rubber o-ring, 3/8-in. inside diameter.

Procedure

1. Preparation of sample. Condition the cigarettes at $75 \pm 2^\circ$ and $60 \pm 2\%$ relative humidity for 24 hours or until the are to be smoked. Determine the average cigarette weight. Select three good cigarettes in the weight range ± 20 mg from the average weight. Reject cigarettes with soft spots or loose ends. Mark each cigarette at the butt length to which it is to be smoked, taking care not to puncture the cigarette paper.
2. Adjustment of smoking machine. Adjust the smoking machine to take a 35 ± 0.5 ml puff of 2 ± 0.2 sec duration every 60 ± 1 sec. The machine should be checked daily and adjusted as necessary.
3. Smoking cigarette. Use a fresh Cambridge filter for each

cigarette.

- a. Attach adapter to rubber balloon with a rubber band.
- b. Attach adapter to solenoid outlet of smoking machine with a short length of Tygon tubing.
- c. Evacuate balloon by puncturing septum on adapter with a hypodermic needle attached to a vacuum line.
- d. Place cigarette in cigarette holder. Anticipate puff by observing sweep second timer on clock.
- e. Have a lighted match ready ~ 2 sec in advance of solenoid actuation. Light cigarette on puff.
- f. Smoke to 23 mm butt length; count the number of puffs.
- g. Both the molecular sieve 5A and the Porapak Q columns are isothermal at 75°C and helium is flowing through the molecular sieve column at 70 cc per min, and through the Porapak column at 30 cc per min.
- h. Puncture septum of adapter with the needle of a 1 ml gas-tight syringe and withdraw 1 ml of smoke from the balloon. Expel the smoke from the syringe and refill it. Repeat three times to obtain sample free of extraneous air.
- i. Inject the sample into the molecular sieve column.
- j. When CO is eluted, refill the syringe as in step "h" and repeat the injection twice.
- k. Refill the syringe as in step "h" and inject the sample into the Porapak column. Repeat procedure twice.
- l. Discard balloon and repeat procedure steps "a" through "k" until three cigarettes of each sample batch have been smoked.

Procedure GP-3 (Preliminary)

Oxides of Nitrogen in the Gas Phase of Cigarette Smoke

Apparatus

1. A smoking machine adjusted to take a 35 cc puff of 2 sec duration once each minute. The machine must be constructed in such a way as to allow each puff to be routed to a partially evacuated trap.
2. Filter holder as described by Ogg⁽¹⁾, etc.
3. Filter disc, CM-113A fiberglass 44 mm diameter from Cambridge Filter Corp., East Syracuse, New York.
4. Rubber membrane, dental dam, 3.5 x 3.5 cm, with 4-6 mm hole in center.
5. Rubber o-ring, 3/8-in. inside diameter.
6. Four 1000 ml capacity wash bottles fitted with a 3 inner joint with an open glass "T" at the top fitted with a stop cock at each end.
7. Cary Model 14 spectrophotometer or Beckman Model B

Reagents

1. N-(1-Naphthyl)-ethylenediamine Dihydrochloride, 0.1%. Dissolve 0.1 gram of the reagent in 100 ml of distilled water.
2. Absorbing reagent. Dissolve 5 grams of sulfanilic acid in ~ a liter of distilled NO₂ free water that contains 140 ml of glacial acetic acid. Add 20 ml of the 0.1% stock solution of N-(1-Naphthyl)-ethylenediamine dihydrochloride and dilute to 1 liter.
3. Reagent grade sodium nitrite. The reagents are stable for several months if they are stored in a cool place away from the light.

Procedure

The cigarettes are conditioned at $75 \pm 2^\circ\text{F}$ and a relative humidity of 60% for 24 hours. A set of four weight-selected cigarettes are smoked one at a time under the standard smoking conditions to the predetermined butt length. Each puff is routed to a partially evacuated trap containing a known volume of absorbing solution. After an hour, the concentration of NO-NO₂ in the gas phase of the cigarette is determined coulometrically as NO₂.

The Collection and Analysis of the Gas Phase of Cigarette Smoke

1. Pipette a 100 ml aliquot of the absorbing solution into the one liter wash bottle.
2. Replace the greased glass inner joint and connect the closed, partially evacuated flask to the discharge side of the smoking machine.
3. Attach a filter-containing filter assembly to the port of the smoking machine.
4. Open the evacuated trap to the discharge side of the smoking machine. The cigarette is isolated from the vacuum by a solenoid valve.
5. Smoke one conditioned cigarette on the smoking machine. Each puff is routed to the evacuated trap during the return stroke of the piston through the now opened solenoid.
6. Remove the cigarette butt from the holder after the cigarette has burned to the 23 mm mark. Take one clearing puff and turn off smoking machine.
7. Close the stopcock on the evacuated flask and disconnect it from the smoking machine.
8. Gently shake the flask. Open the stopcock on the flask to allow its contents to come to atmospheric pressure and quickly close the stopcock.
9. Gently shake the flask again for ~ one minute.
10. If one chooses, the above 1 through 9 steps may be repeated until the gas phase of all four smoked cigarettes has been collected in four individual flasks containing 100 ml each of the absorber solution before proceeding further with the analysis.

Analysis of Absorber Solution(s)

1. After the absorber solution(s) have stood for at least one hour in the flask(s), determine the absorbance of each solution with the spectrophotometer at 550 m μ using the absorber reagent as the reference solution.
2. Compare these respective readings to a calibration curve to obtain the total NO₂ present in the absorber solution.

3. If the absorbance reading of a sample is too high to be read on the calibration curve, dilute an aliquot two-fold with absorbing reagent. Again proceed with steps 1 and 2.

Preparation of a Calibration Curve

1. Prepare a 100 mg/ml NO_2 standard solution by accurately weighing 0.150 grams of reagent grade sodium nitrite. Dissolve the salt in distilled water and transfer to a liter volumetric flask. Dilute to the mark with distilled water.
2. Pipette 0.25 ml, 0.50 ml, 0.75, 1.0 ml, 1.5 ml, and 2.0 ml aliquots of the standard solution into separate 100 ml volumetric flasks. Dilute to the mark with the absorbing reagent. Wait at least 15 minutes then determine the absorbance of the standard colored solutions at 550 m μ on a spectrophotometer using the reagent blank as the reference solution.
3. Construct a calibration curve by plotting the NO_2 content (in micrograms) present in each standard solution versus the respective absorbance reading.

References

1. Ogg, C.L., J.A.O.A.C 47, 356 (1964)
2. Newsome, J. R., Norman, V., and Keith, C. H., Tobacco Sci. 9, 105 (1965)
3. Morie, G., Tennessee Eastman, private communication, 1970.

Procedure GP-4 (Preliminary)

Determination of Formaldehyde in the Gas Phase of Cigarette Smoke

Apparatus

1. Smoking machine capable of drawing one puff of 35 cc volume in two sec once per min. Glass fiber "Cambridge" filters held in standard plastic filter assemblies equipped with latex dental dam cigarette holders.
2. Smoke trap constructed by attaching a glass cylindrical receiver 30 mm in diameter and 150 mm long to the bottom of an 800 ml Kjeldahl flask using a 34/45 standard-taper joint. Smoke is routed from the Cambridge filter into a trapping solution contained in the receiver.
3. Spectrophotometer-Cary Model 14 or Beckman Model B.
4. 12-20 x 200 mm glass test tubes.

Reagents

1. Sodium Metabisulfite Trapping Solution. Prepared by dissolving 10 grams of sodium metabisulfite in one liter of water.
2. Chromatropic Acid Solution. Dissolve 10 grams in 100 ml of water in a one liter volumetric flask. Slowly, with cooling, dilute to the mark with concentrated sulfuric acid (this dilution should be made over a period of approximately one hour). Store the resulting solution in the dark.
3. Formaldehyde.

Procedure

Cigarette handling, conditioning, selection and smoking are carried out in the standard manner. Three cigarettes are smoked individually through Cambridge filters into the trap solution. Four sets of three cigarettes each are smoked for this analysis. New filter pads are used for each cigarette smoked. Upon completion of smoking, the trapping solution is removed and analyzed colorimetrically for formaldehyde content.

Generation and Collection of the Gas Phase Sample

1. Pipette 50 ml aliquots of the stock 1% sodium metabisulfite absorbing solution into each of four receivers. Attach one receiver to each of four modified Kjeldahl flasks mounted behind the cigarette ports.
2. After placing new Cambridge filters in each of the four filter assemblies, smoke one conditioned, weight-selected cigarette at each port. The cigarettes are smoked to 23 mm butt lengths using one 35 cc puff of 2 sec duration once per min in a room maintained at 75°F and 60% relative humidity.
3. Repeat the smoking process with two additional cigarettes through each absorbing solution. Change filters after each cigarette is smoked. It is advisable to incorporate one clearing puff after each cigarette is removed from the filter assembly.
4. Allow the receivers containing the absorbing solution to stand at least 10 min before detaching them from the Kjeldahl flasks and proceeding with the analysis.

Analysis of Smoke Solutions

1. Pipette 2.0 ml aliquots of each smoke solution into individual tubes. Pipette a 2 ml aliquot of the 1% sodium metabisulfite solution into a test tube to serve as a reagent blank.
2. Add 10.0 ml of the chromotropic and reagent to each of the test tubes. Mix and place the tubes in a boiling water bath.
3. Remove the test tubes after 30 min and cool to room temperature.
4. Determine the absorbance of each solution with the spectrophotometer at 575 m μ and 700 m μ using the reagent blank as the reference solution.
5. Determine $A_{575} - A_{700}$ reading for each solution. Compare these respective net readings to a calibration curve to obtain the formaldehyde present in the 2.0 ml aliquot.

Preparation of a Calibration Curve

1. Accurately weigh an aliquot of the analyzed 37% reagent grade formaldehyde solution needed to prepare a 100 μ g/ml formaldehyde standard when this weight is diluted to a 1000 ml volume with an aqueous solution of 1% sodium metabisulfite.

2. Pipette 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, and 5.0 ml aliquots of this standard solution into five separate 100 ml volumetric flasks. Dilute each standard to 100 ml with 1% sodium metabisulfite.
3. Pipette 2.0 ml of each of the five solutions into five separate test tubes.
4. Treat these standards in the same way the samples are treated. Construct a calibration curve by plotting the formaldehyde content (in micrograms) present in each test tube versus the respective 575 mμ-700 mμ net absorbance reading obtained with the standard solutions using the reagent blank as a reference solution.

References

1. Newsome, J. R., Norman, V., and Keith, C.H., Tobacco Sci. 9, 102 (1965)

Procedure GP-5 (Preliminary)

Determination of Hydrogen Cyanide in the Gas Phase of
Cigarette SmokeApparatus

1. Spectrophotometer - Range 325-1000 millimicrons
2. Cuvettes
3. Pipettes
4. 5" glass tubes - 9 mm o.d. - 7 ml i.d.
5. Clinical Centrifuge
6. Smoking machine

Reagents

1. Ascarite 8-20 mesh
2. Bromine-saturated water
3. Sodium arsenite - 2% in water
4. Pyridine solution (25 ml pyridine, 73 ml H_2O + 2 ml conc. HCl).
5. Phenylenediaminedihydrochloride solution (2 g P-phenylenediamine dissolved in 98 ml H_2O + 3 ml conc. HCl).
6. 1.0 molar sodium hydroxide
7. HCl-water (1:1)

Procedure for Trapping and Recovery of HCN

1. Fill glass tube with 3 g of ascarite and put a small plug of glass wool in each end.
2. Attach glass tube to smoking machine.
3. Attach Cambridge filter holder to ascarite tube and smoke two cigarettes, to a 23 mm butt length, changing Cambridge filter between cigarettes. The ascarite tubes may then be stored in a tightly sealed bottle, but should be analyzed within three hours.
4. Empty contents of glass tube into a 100 ml beaker and add 30 ml H_2O .
5. Let stand 15 min, stirring occasionally.
6. With a small amount of water, rinse the mixture into a 50 ml centrifuge tube and centrifuge at high speed for approximately 1 min. Decant through a small funnel, containing a small pad of glass wool, directly into a 100 ml volum flask. Rinse the

beaker again, into the centrifuge cone, stirring precipitate in cone, and centrifuge again. Rinse the precipitate three more times with ~ 10 ml water and add to 100 ml flask. Dilute to volume. This solution should be analyzed within one hour.

Procedure for Analysis

1. Pipette a 1 ml aliquot into a 25 ml volumn flask; add 7 drops of 1:1 HCl.
2. Add 4 drops of saturated bromine water, and then destroy excess bromine with 5 - 6 drops of sodium arsenite solution.
3. Add 2 ml P-phenylenediamine solution, and 2 ml pyridine solution.
4. Let the color develop 7 - 10 min, and dilute to the mark with distilled water. Read at 515 m μ on spectrophotometer.

Note: The samples should be read immediately after diluting as the color will continue to intensify and erroneous results will be obtained.

5. Make a blank containing all reagents except bromine water.
6. Set the meter at 100% transmittance and read % transmittance for samples.
7. Read micrograms of CN⁻ from calibration curve.

Preparation of Calibration Curve

Dissolve 98 mg of sodium cyanide in a liter of water. This gives a concentration of 52 μ g of cyanide per ml. From this solution pipette 2, 5, 10, and 20 ml aliquots into four 100 ml flasks and dilute to volume with 0.5 M NaOH. Use 1 ml aliquots from each dilution and develop color as previously described. Read % transmittance and plot on semilog paper. Concentration of final dilutions is 1.04 μ g, 2.60 μ g, 5.20 μ g, and 10.40 μ g CN⁻/ml.

Calculation:

$$\mu\text{g HCN/cig} = \mu\text{g CN}^- \times \frac{100}{\text{number of cigarettes} \times \text{aliquot}}$$

References

1. Analysis of Hydrogen Cyanide in Whole Cigarette Smoke (Tennessee Eastman Research Laboratories) Procedure 27 (Revised) 3-3)70 (CHS).
2. "A New Method for the Estimation of Micro Quantities of Cyanide and Thiocyanide," W. N. Aldridge, Analyst 69:262, 1944.

3. "Investigation of Reagents for the Colorimetric Determination of Small Amounts of Cyanide," L. S. Burk and H. G. Higson, Talanta, Vol 11: 471-479, 1964.
4. "An Automated Method for Determination of Hydrogen Cyanide in Cigarette Smoke," Tobacco Science, XIV, 12-15, January 16, 1970.

Procedure GP-6 (Preliminary)

pH of the Gas Phase of Cigarette Smoke

Apparatus

1. A smoking machine that is adjusted to take a 35-cc puff for a 2-second duration once each minute.
2. Filter holder as described by Ogg⁽²⁾ consisting of threaded inner and outer parts and neoprene gasket.
3. Filter disc, CM 113A Figerglass, 44 mm diameter, from Cambridge Filter Corp., East Syracuse, New York.
4. Rubber membrane, dental alum 3.5 x 3.5 cm, with 4-6 mm hole in center.
5. Rubber O-ring, 3/8 inch inside diameter.
6. A combination smoke collection chamber and electrode holder.
7. Heath pH recording electrometer or an equivalent type pH recording device with an expanded scale feature.
8. Electrode, Beckman combination electrode No. 39183 (modified).⁽¹⁾

Reagents

1. Buffer solutions, pH 4 and 6

Procedure

The cigarettes are conditioned at $75 \pm 2^{\circ}\text{F}$. and a relative humidity of $60 \pm 2\%$ for at least 24 hours. Four cigarettes are selected in the weight range of ± 20 mg from the average weight. The pH of the gas phase of the cigarette is recorded as the smoke is drawn through a filter and comes in contact with a buffer conditioned electrode.

Analysis of the gas phase of cigarette smoke

1. Connect the electrode to the pH meter.
2. Turn ON the pH meter.
3. Set the meter on the expanded scale of 2.
4. Set the lower pH index knob at 5.
5. Standardize the pH meter with pH 7.0 buffer solution.
6. Remove the electrode from the buffer solution.
7. Wash the electrode with distilled water and wipe it with tissue paper.
8. Set the lower pH index knob at 4.

9. Place the electrode in pH 4.0 buffer solution to check the accuracy of the pH meter.
10. Remove the electrode from the pH 4.0 buffer solution.
11. Wash the electrode with distilled water and wipe it with tissue paper.
12. Reset the lower pH index knob at 5.
13. Dip the tip of the electrode into the pH 7.0 buffer solution.
14. Remove the electrode and wipe the sides with tissue paper.
15. Gently blot the tip of the electrode with tissue paper so that only a thin film of the buffer solution remains.
16. Place the electrode into the top of the smoke collection chamber.
17. Place the preassembled filter assembly unit into the bottom of the smoke collection chamber. The exit of the filter assembly unit is positioned approximately 1-1/2 inches beneath the tip of the electrode.
18. Connect the side arm of the smoke collection chamber to the smoking machine.
19. Activate the recorder and fix a reference point at a pH of 6.5.
20. Set a conditioned cigarette into the filter holder.
21. Activate the smoking machine.
22. Light the cigarette as the machine takes the first puff.
23. Record the pH during the smoking cycle of the cigarette. During the smoking cycle the lower index knob may have to be switched to 4 and sometimes to 3.
24. After the cigarette is smoked to the desired butt length remove it from the filter holder.
25. Turn off the power supply to the smoking machine and recorder.
26. Remove the electrode from the smoking chamber.
27. Wash the electrode with distilled water and dry it with tissue paper.

Treatment of Data

1. The minimum and maximum pH readings are tabulated for the final 4 to 10 puffs of the smoke cycle of the cigarette.
2. The pH of the gas phase of the smoke of at least three cigarettes of a given sample will be determined on a puff by puff basis.

Reference

1. Sensabaugh, A. J. and Cundiff, R. H., Tobacco Sci. 11, 25 (1967).

Procedure C-1 (Preliminary)

Determination of Water and Acetone in Smoke Condensate Preparations

Apparatus

1. Gas chromatograph, MicroTek No. 160. Column: 3' x 0.25" copper packed with 80-100 mesh Porapak Q. Column temperature = 180°C, Inlet temperature = 200°C, Detector temperature = 210°C, Helium carrier gas flow rate $\approx$ 80 ml/min, Thermal conductivity detector current = 200 ma, Attenuator = 32.
2. 3.5 milliliter capacity screw-topped vials with teflon-faced diaphragm caps.
3. Precision gas chromatography syringes.
4. Vortex mixer.

Reagents

1. 100:1 dry dioxane - absolute ethanol

Procedure

At least three 100 mg samples of the acetone-water suspension of a condensate are to be analyzed. These samples are contained in 3.5 milliliter capacity diaphragm capped vials.

Analysis of the samples

1. Accurately deliver 2.0 ml of 100:1 dioxane: isopropanol to each sample-containing vial.
2. Mix vigorously with a vortex mixer and allow the samples to stand at least 30 minutes.
3. Inject at least three 5 μ l aliquots of each sample into the gas chromatograph. Similarly inject 5 μ l aliquots of the extracting solvent as a blank.
4. Measure the heights of the peaks corresponding to water, ethanol, and acetone. Determine the ratios of the peak heights water/ethanol and acetone/ethanol. Average the ratios obtained from multiple injections of a given sample. Subtract the ratio determined for the blank from those for the samples. Compare these blank-corrected ratios to a calibration curve.

Preparation of a calibration curve

1. Draw 2.0 ml of the dioxane-ethanol into each of six vials of the type used for the samples. Add the following to five of the vials with calibrated precision syringes: 2.5 μ l H₂O + 5.0 μ l acetone, 50 μ l H₂O + 15.0 μ l acetone, 10.0 μ l H₂O + 25.0 μ l acetone, 15.0 μ l H₂O + 40.0 μ l acetone, and 20.0 μ l H₂O + 50.0 μ l acetone. The sixth vial serves as a reagent blank for both the standards and the samples.
2. Treat the standards in the same way the samples are treated. Construct a calibration curve by plotting the blank-corrected H₂O/ethanol and acetone/ethanol peak height ratios versus the water and acetone contents, in milligrams, of the vials.

Procedure C-2 (Preliminary)

Determination of Nicotine and Nornicotine in Condensate

Apparatus

1. Gas chromatograph, MicroTek No. 160 or equivalent. Column: 6' x 0.25" stainless steel packed with 5% Casterwax on acid washed, DMCS-treated 80-100 mesh Chromosorb G. Column temperature = 170°C, Inlet temperature = 180°C, Detector temperature = 210°C, Helium carrier gas flow rate = 80 ml/min, Hydrogen Flame Ionization Detector.
2. Thirty-five milliliter capacity screw-topped vials with teflon-faced diaphragm caps.
3. Twenty-five microliter precision syringe.
4. Vortex mixer.

Reagents

1. Methanol containing 0.4 mg/ml dimethylphthalate.

Procedure

At least three 100-mg samples of the acetone-water suspension of a condensate are to be analyzed. These samples are contained in 3.5 ml capacity diaphragm capped vials.

1. Transfer the sample into a 35-ml diaphragm capped vial using 10 ml of methanol containing 0.4 mg/ml of dimethylphthalate.
2. Stir the capped solution with a Vortex mixer for one minute and allow to stand for at least one hour.
3. Inject 10 µl aliquots of the solution into the gas chromatograph. Note the heights of the peaks for nicotine, nornicotine and dimethylphthalate. Compare the peak-height ratios of nicotine/dimethylphthalate and nornicotine/dimethylphthalate to a previously prepared calibration curve.

Preparation of a calibration curve

1. Prepare standards containing 2, 4, 6, and 8 mg of nicotine and 0.2, 0.4, 0.6, and 0.8 mg of nornicotine in 10 ml of methanol containing 0.4 mg/ml of dimethylphthalate.

2. Inject 10 μ l aliquots of each into the chromatograph and plot the ratios of the peak heights nicotine/dimethylphthalate and nornicotine/dimethylphthalate versus the concentration of each alkaloid.

Reference

Mumpower, R. C. and Kiefer, J. E., Tobacco Science 11, 144 (1967).

Procedure C-3 (Preliminary)

Nicotine Alkaloids in Smoke Condensate

Apparatus

1. Griffith steam distillation apparatus, six port unit from Lab Glass, Inc., Kingsport, Tennessee.
2. Six, 250 ml short form (Pyrex), wide mouth, flat bottom volumetric flasks.
3. Spectrophotometer, Cary Model 14 or equivalent.
4. Magnetic stirring motor and Teflon-coated stirring bar (1-in. x 1/2-in.).

Reagents

1. Hydrochloric acid, ~ 36% reagent grade for the preparation of a 0.2 N solution, and 1 + 9 HCl + H₂O.
2. Sodium hydroxide-Sodium chloride solution. Dissolve 300 grams NaOH in distilled water and dilute to one liter. Saturate with NaCl and cool. Stir and after 24 hours, decant the supernate.
3. Nicotine purified by vacuum distillation. Prepare a standard solution by weighing one gram and diluting with 0.05 N HCl to 100 ml. Prepare fresh each 30 days.

Analyses of Samples

1. Prepare the Griffith Still for operation.⁽¹⁾
2. Pipette a 5 ml aliquot of 0.2 N HCl into the respective still pots.
3. Quantitatively transfer a sample of the condensate into each of the still pots. Pipette no more than a one ml aliquot of a 10 µg/ml nicotine standard into one still pot that contains 5 ml of the 0.2 N HCl solution.
4. Steam distill each acid solution until ~ 100 ml of distillate is collected.

5. Discard this distillate and place a short form, wide mouth, 250 ml volumetric flask containing 10 ml of 1:9 HCl:H₂O under each U tube receiver.
6. Add 5 ml of the sodium hydroxide-saturated sodium chloride solution to each still pot and steam distill until ~ 230 ml of distillate is collected in each flask.
7. Dilute each distillate to the 260 ml (pre-marked) line on the flask with distilled water and equilibrate each solution.
8. Measure the absorbance of each distillate at three wave lengths; 236, 259, and 282 mμ respectively with a spectrophotometer using a 0.05 N hydrochloric acid solution as the reference blank.

Calculations

The concentration of nicotine in each distillate is calculated in the following manner:

$$1. A'_{259} = 1.059 \times [A_{259} - 1/2 (A_{236} + A_{282})]$$

Where A = The absorbance at the subscripted wave length.

And A'_{259} = Background-corrected absorbance at 259 mμ.

$$2. \frac{A'_{259}}{a} \times V = \text{mg of nicotine in distillate}$$

Where V = Volume of distillate times dilution factor (if any).

a = A/(c x b) (accepted value = 34.3).

A = Absorbance reading of a standard nicotine solution at 259 mμ.

c = Concentration of nicotine in 0.05 N HCl in g/l.

b = Cell length.

References

1. Griffith, R. B., Tobacco Sci. 1, (130-137) 1957.

Procedure C-4 (Preliminary)

Determination of Phenol, o-Cresol and m-Cresol plus p-Cresol in Condensate

Apparatus

1. Gas chromatograph, MicroTek No. 160 or equivalent. Column: 6' x 0.25 stainless steel packed with 5% (w/w) Casterwax on acid-washed DMCS-treated 80-100 mesh Chromosorb G. Column temperature = 140°C , Inlet temperature = 150°C , Detector temperature = 175°C , Helium carrier gas flow rate = 80 cc/min, Hydrogen flame ionization detector.
2. Griffith steam distillation apparatus.
3. Separatory funnels.
4. Calibrated, capped, 15-ml centrifuge cones.

Reagents

1. Anhydrous diethyl ether
2. Scintillating solution: 4 g of 2,5-diphenyloxazole and 50 mg of p-bis(2-(5-phenyloxazolyl))benzene per liter of toluene.
3. Phenol, o-Cresol, m-Cresol, p-Cresol and Phenol- ^{14}C .
4. Sodium bicarbonate, sodium chloride, sodium hydroxide, hydrochloric acid, anhydrous magnesium sulfate.
5. Cylinder of dry nitrogen gas.

Procedure

At least three 100-mg samples of the acetone-water suspension of a condensate are to be analyzed. These samples are contained in 3.5 milliliter capacity diaphragm capped vials.

Analysis of the samples

1. Add a known quantity of phenol- ^{14}C to the sample and transfer the sample to the still pot of the Griffith distillation apparatus using 5-10 ml of 0.2 N HCl.
2. Distill from HCl until 250 ml of distillate is collected.
3. Add 2 ml of 2 N NaOH and extract the distillate with 2 100-ml portions of anhydrous diethylether.

4. Acidify the aqueous layer, neutralize with solid sodium bicarbonate and saturate with sodium chloride.
5. Extract the neutralized layer with 3 50-ml portions of anhydrous diethylether.
6. Dry the combined extracts over anhydrous magnesium sulphate and allow to stand for 2 hours. Filter.
7. Cool the extract in a refrigerator and evaporate to 10 ml by blowing dry nitrogen over the extract.
8. Inject 10 μ l aliquots of the solution into the gas chromatograph. Subject a portion of the final solution to liquid scintillation counting.

Preparation of a calibration curve

1. Make standards containing phenol, o-cresol, m-cresol and p-cresol in methanol. Weigh enough of each phenol to yield solutions containing all of the phenols in the range of 0.01-0.10 μ g/ μ l
2. Inject aliquots into the chromatograph and plot the peak areas on peak heights versus the known concentration of phenols.

Reference

Oakley, E. T., Millham, J. O. and Weissbecker, L., Analytica Chimica Acta 31, 272-278 (1964).

Procedure C-5 (Preliminary)

Phenol Number of the Condensate

Apparatus

1. Griffith distillation apparatus, six port unit available from Lab Glass, Inc., Kingsport, Tennessee.
2. Cary Model 14, spectrophotometer.
3. Twelve, 250 ml, Teflon-lined, screw cap, separatory funnels.
4. Three, 250 ml, wide mouth, flat bottom volumetric flasks.
5. Twelve, 50 ml beakers.
6. Twelve 50 ml volumetric flasks.

Reagents

1. Four aminoantipyrene (Matheson, Coleman and Bell).
2. Potassium ferricyanide (Baker), prepare an aqueous 10% solution.
3. Chloroform, reagent grade (Mallinckrody).
4. Three N ammonium hydroxide.
5. 0.2 N hydrochloric acid.
6. Phenol: reagent grade (Baker).

Procedure

At least three 100 mg samples of the acetone-water suspension of a condensate are to be analyzed. The samples contained in 3-5 milliliter capacity diaphragm-capped vials and quantitatively transferred into separate still pots and steam distilled from an acidic medium. After the addition of the reagents to aliquots of each distillate, several extractions with chloroform are carried. The absorbance of each sample's combined extract are measured at 454 m μ with a spectrophotometer. The phenol number will be determined from a calibration curve prepared from the absorbance readings given by pure phenol standards.

Analysis of Samples

1. Quantitatively transfer each of the samples of condensate to separate still pots of the Griffith distillation apparatus using no more than 5 ml of distilled water for each transfer.

2. Add 5 ml of 0.2 N HCl to each still pot.
3. Steam distill until a volume of ~ 230 ml of distillate is collected. Dilute the distillate to 250 ml in the flask and equilibrate.
4. Pipette a 100 ml aliquot of each distillate into three individual 250 ml separatory funnels. Add 100 ml of distilled water to another funnel and treat in the same manner as the samples.
5. To each funnel add 0.3 ml of the 3 N ammonium-hydroxide, 0.7 ml of the 3% 4-aminoantipyrine solution and 0.7 ml of the 10% potassium ferricyanide solution. Swirl each funnel after the addition of each respective reagent.
6. ~ Ten ml of reagent grade chloroform is added to each funnel and each solution is shaken for a two min period.
7. After the chloroform phase has separated, draw off each extract into a 50 ml beaker.
8. Repeat steps 6 and 7 with fresh chloroform until three extractions have been completed.
9. Transfer each combined sample extract to a 50 ml volumetric flask, dilute to the mark with chloroform, and equilibrate.
10. Measure the absorbance of each combined red colored extract at 454 mμ against the reagent blank extract with a spectrophotometer.
11. Compare the blank-corrected reading of each of the extracts to a calibration curve to determine the phenol number for each of the condensate sample aliquots.

Preparation of a Calibration Curve

1. Prepare a 100 μg/ml standard aqueous phenol solution. Accurately weigh 100 mg of phenol. Transfer the reagent to a liter volumetric and dilute to the mark with distilled water.
2. Put ~ a 100 ml of distilled water in each of six separatory funnels.
3. Pipette a 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, and 5.0 ml aliquot respectively into each funnel. Reserve one funnel for a reagent blank.

4. Treat these standards the same way the samples are treated. Construct a calibration curve by plotting the phenol content (in micrograms) of each combined extract versus their respective blank-corrected absorbance readings.

References

1. Oakley, Elizabeth, T., Mullham, J. O., and Weisbecker, L., Anal. Chem. Acta. 31, 275-276 (1964).

Procedure C-6 (Preliminary)

Determination of Phenanthrene, Benz(a)anthracene,
and Benzo(a)pyrene in Tobacco Smoke CondensateApparatus

1. Freezer, magnetic stirrer, weighing vials, 15 ml graduated screw cap centrifuge cones, 125 and 250 ml separatory funnels.
2. Chromatographic glass sandwich plates and clamps, jars and accessories. Micropipettes. Alumina column 2 cm diameter x 45 cm tall, reservoir stopcock, packing frits and accessories. Supplied by Chromaflex Chromatographic Apparatus, Kontes, Vineland, New Jersey.
3. Beta-gamma scintillation counter, standards and accessories. Packard Model 3002, Tri Carb Scintillation Spectrometer, Packard Inst. Co. Inc., 2200 Warrenville Road, Downers Grove, Illinois 60515.
4. Perkin-Elmer Fluorescence Spectrophotometer 203, The Perkin-Elmer Corp., Norwalk, Connecticut 06852.
5. Kensco Mineralight, Kensington Scientific Corp., Oakland, California 94608.

Reagents

1. Methanol, acetone, hexane, cyclohexane, and benzene of nanograde quality. Mallinkrodt Chemical Works, St. Louis, Missouri 63160.
2. Dimethyl sulfoxide, nitrobenzene (purified by distillation if necessary), J. T. Baker Chemical Co.
3. Nitrogen gas and regulators.
4. Woelm Aluminum Oxide, neutral, activity II, Waters Associates Inc., 61 Fountain St., Framingham, Massachusetts 01701.
5. PPO and POPOP scintillator chemicals, Packard Inst. Co., Inc., Downersgrove, Illinois 60615.
6. Phenanthrene, benz(a)anthracene, and benzo(a)pyrene, Aldrich Chemical Co., Inc., 940 West St., Paul Avenue, Milwaukee, Wisconsin

53233. The same chemicals tagged with radioactive carbon-14 may be prepared on Custom Contract by Amersham Searle Corp., 2636 S. Clearbrook Drive, Arlington Heights, Illinois 60005, phone (312) 296-1055. Also from Mallinkrodt-Nuclear, St. Louis, Missouri 63160.

7. Chromatography and Electrophoresis Paper, grade 2495 acetylated cellulose, size 54 x 55 cm, Carl Schleicher and Schuell Co., Keene, New Hampshire.

Procedure

1. Remove the condensate sample from the freezer and let thaw for two hours while stirring on a magnetic stirrer.
2. While continuing to stir, use a pasteur pipette to remove 5-7 portions, and place in a previously weighed vial.
3. Weigh the vial to determine the amount of sample taken for analysis. (The sample weight should be equivalent to 2-3 g of dry condensate).
4. Add 5 ml of methanol:water (4:1) to sample.
5. If carbon-14 isotopes are to be used as internal standards, appropriate aliquots should be added at this point.
6. Using a pasteur pipette transfer the sample to a 125 ml separatory funnel. Rinse the pipette and vial with three 10 ml portions of methanol:water (4:1), and add each to the separatory funnel. Swirl and shake to allow the sample to digest in this solvent. Pre-equilibrate the methanol:water solution with cyclohexane.
7. Rinse the vial with two 20 ml portions of cyclohexane (nanograde or previously purified by distillation), and add each to the separatory funnel.
8. Extract⁽¹⁾ the methanol:water phase for 5-10 min by equilibrating on a mechanical shaker.
9. Allow the phases to settle for 5-10 min before separating.
10. Carefully transfer the lower phase to another separatory funnel.
11. Add 40 ml of cyclohexane to the second funnel containing the methanol:water phase; repeat steps 3 and 4.

12. Transfer the lower phase to a third funnel, and transfer the cyclohexane phase to the first funnel.
13. Extract the methanol:water phase a third time by repeating step 6.
14. When the three cyclohexane fractions have been combined in a funnel, carefully drain off the last traces of methanol:water phase; wash this phase with cyclohexane, 5-10 ml, and add to the cyclohexane funnel.
15. Evaporate the cyclohexane down to a volume of 20 ml by reducing the pressure above the solution with the lab vacuum system and allowing a gentle stream of nitrogen to sweep the vapor from the system.
16. If the cyclohexane was transferred to a flask for evaporation, the residual solution must be transferred to a 125 ml separatory funnel; rinse the flask with enough cyclohexane to give a total volume of 40 ml.
17. Extract the cyclohexane solution for 5-10 min with 10 ml of DMSO⁽²⁾. Allow the phases to settle for 10 min. Transfer the DMSO layer to a 250 ml separatory funnel.
18. Repeat the extraction with DMSO for a total of 7 times. The total volume of DMSO extract should be 70 ml.
19. Slowly add 140 ml of distilled water to the funnel containing the DMSO extract. When approximately 100 ml of water has been added, the DMSO layer will turn from a clear dark brown color to a turbid light brown color.
20. Extract the solution with a 30 ml portion of cyclohexane. The time required for the phases to settle may be as long as two hours since this mixture tends to emulsify.
21. Carry out a total of four extractions with cyclohexane. The total volume collected will be 120 ml.
22. Transfer the cyclohexane to an evaporation flask; rinse the funnel with 2-3 portions of cyclohexane, and reduce the volume down to approximately 5 ml.

23. Transfer the cyclohexane to a tapered, graduated, 15 ml centrifuge cap with a screw top. Further evaporate the solution to two ml. The sample is now ready for column chromatography on alumina.
24. Transfer the sample to the head of an alumina column^(a).
25. Open the stopcock and collect the effluent in 50 ml fractions.
26. Add one 5 ml fraction of hexane to wash the sample onto the alumina bed.
27. Fit the column with a reservoir containing 295 ml of hexane and start adding this to the column as soon as the 5 ml is absorbed into the alumina bed. Add all the hexane, but do not allow the alumina bed to start going dry.
28. While the last few ml of hexane is going onto the column, fill the reservoir with 300 ml of 8:1 hexane-benzene solution and add this to the column immediately behind the hexane.
29. After all the 8:1 hexane-benzene has been added, pass 600 ml of 6:1 hexane-benzene over the column in a similar way.
30. After the last fraction has been collected, remove 1 ml from each fraction and add it to 9 ml of scintillator solution in a 20 ml scintillator vial^(b).
31. Determine the amount of radioactivity in each fraction by gamma counting.
32. Plot the total activity in counts per min versus the effluent volume in ml for each fraction on 4 or 5 cycle semilog graph paper.
33. The first peak is phenanthrene; the second is benz(a)anthracene, and the third is benzo(a)pyrene.
34. Transfer all fractions that fall under the phenanthrene peak into an appropriate container and evaporate (c) the solution to 0.2 ml or less so that it may be transferred onto a paper chromatography sheet, label, 1a.
35. Treat the fractions that fall under the benz(a)anthracene in a similar way, and also the fractions under the benzo(a)pyrene peak, label 1b and 1c.

36. Prepare a paper chromatogram as follows: Cut acetylated cellulose paper into 20 x 20 cm square sheets. Mark the sheets lightly with a carbon pencil 3 cm from one edge. This mark will be the sample starting line. Beginning at the left, put light pencil marks at the following intervals on the starting line; 2, 6, 8, 10, 12, 14, 16, and 18 cm. Place the sheets in a large dessicator, containing drierite, for at least 24 hours before usage.
37. Prepare three sets of dry chromatographic glass sandwich plates with clamps. Label the sets 1a, 1b, and 1c with lead pencil.
38. Pipette 10 ml of standard solution containing 1 mg each of phenanthrene, benz(a)anthracene, and benzo(a)pyrene onto the starting line 2 cm from the left edge of a paper chromatographic sheet. Pipette about 10 ml portions beginning 6 cm from the left edge and adding another portion at every 2 cm interval on the starting line. If necessary, allow the additions to dry about 2 min and add more sample on top of them.
39. Secure the paper chromatogram between the two glass plates with clamps. Place the "sandwich" in the developer jar with the starting edge down.
40. Allow to develop until the developer front is 1 cm from the top of the chromatogram. Development time is about 2.5 to 3 hours.
41. Remove the chromatogram from the developer and sandwich and hang up to dry by the starting edge for 10 min at room temperature.
42. Examine the chromatogram in a black box under ultraviolet light at 254 and 366 m μ wavelength, use the mineralight.
43. Mark the outline of the phenanthrene, benz(a)anthracene, and benzo(a)pyrene on their respective chromatograms using the standards as a guide.
44. Cut out the outlined compounds with scissors and treat each compound separately. Avoid cross contamination.
45. Elute or extract the compound from the paper with benzene.

46. Filter the solution through a small column of alumina using benzene as a washing agent.
 47. Catch the filtered solution in a 15 ml centrifuge cone, and evaporate the solution down to less than 5 ml.
 48. Make the solution up to 5 ml in a volumetric flask with benzene.
 49. Make up standards for each compound in benzene.
 50. With a fluorimeter, determine the fluorescence of the standards and the compound in question. Dilute the compound solution if necessary with benzene.
 51. Plot the concentration of the standards versus their fluorescence on linear graph paper.
 52. Read the unknown concentration of the compound from the standard curve and calculate the total quantity of the compound present.
-
- (a). The alumina column is 45 cm tall, 2 cm in diameter and filled with 30 cm of Woelm aluminum oxide, neutral, activity II, in hexane. It takes about 196 g of the dry aluminum oxide for one column. The column is fitted with a stopcock to regulate the flow rate and a reservoir for adding column elutants.
 - (b). Prepare a scintillator solution containing 50.0 mg of POPOP and 4.00 g of PPO in 1 liter of toluene.
 - (c). Evaporate the combined effluent fractions under reduced pressure with a gentle stream of nitrogen gas passing over them. The fractions for each compound should first be combined in a 500 ml modified wash bottle fitted with a ground glass joint containing an inlet for a nitrogen stream and a connection for vacuum. After the volume is reduced to about 5 ml, transfer the solution to a 15 ml centrifuge cone and evaporate to about 0.2 ml or less, but do not evaporate to dryness since the dry product is usually difficult to redissolve. About 25 to 50 ml is appropriate for transfer to a paper chromatographic sheet.

References

1. Tobacco and Tobacco Smoke, Ernest L. Wynder and Dietrich Hoffman, Academic Press, 1967.
2. Dimethyl Sulfoxide: A Superior Analytical Extraction Solvent for Polynuclear Hydrocarbons and for Some Highly Chlorinated Hydrocarbons, Edward O. Haenni, John W. Howard, and Frank L. Joe, J.A.O.A.C., Vol. 45, No. 1, 1962.

Procedure C-7 (Preliminary)

Weak and Very Weak Acids of Smoke Condensate

Apparatus

1. Potentiograph E-336 or equivalent fitted with a 10 ml microburet available from Brinkmann Instruments, Westbury, New York.
2. Glass electrode, Beckman No. 4990-80 or equivalent.
3. Beckman sleeve-type calomel electrode No. 1170-71 or equivalent, modified by replacing the saturated aqueous potassium chloride solution in the outer jacket with a saturated solution of potassium chloride in methanol.
4. Four - 100 ml electrolytic Pyrex beakers.
5. Argon gas.
6. Magnetic-stirring motor and 1-in. x 1/4in. Teflon-coated magnetic stirring bar.

Reagents

1. 0.02 N methanolic tetrabutylammonium hydroxide. Add 30 ml of methanol to 200 ml 0.1 N tetrabutyl ammonium hydroxide and dilute to one liter with benzene.
2. Pyridine. Allow to stand 16 hours over sodium hydroxide. Flush distill and sparge the distillate for 10 min with argon. Store in tightly closed bottle until used.
3. 3,5 dinitrosalicylic acid and 0.1 gram benzoic acid in pyridine and dilute to 250 ml with pyridine.

Procedure

At least three 100 mg samples of the acetone-water suspension of condensate are to be analyzed. These samples are contained in a 3.5 milliliter capacity diaphragmed capped bottle.

Analysis of Condensate Samples

1. Transfer a 100 mg sample of the condensate to a beaker containing 20 ml of pyridine and 10 ml of the standard 3,5 dinitrosalicylic-benzoic acid spike solution. Rinse the vial with two-2.5 milliliter aliquots of pyridine.

2. Sparge the solution with argon for 10 minutes.
3. While stirring, potentiometrically titrate the solution under argon with 0.02 N tetrabutylammonium hydroxide. (It is best to sparge the titrant prior to the first titration of the day.)
4. Perform a blank analysis by titrating 10 ml of the dinitro-salicylic acid-benzoic acid solution and 25 ml pyridine in a similar manner.

Standardization of Titrant

1. Add 10 ml of 0.1 N standardized acid to a beaker containing 35 ml of pyridine.
2. Sparge with argon for 10 min and potentiometrically titrate the acid solution under the described sample analysis conditions.
3. Determine the equivalence point from the potentiometric curve.

Calculations

1. Determine the weak and very weak acid equivalence points from each of the potentiometric curves.
2. Subtract the weak and very weak acid blank analysis equivalence points from the respective condensate plus blank equivalence points.
3. Determine the net milli-equivalents of weak and very weak acids present in the condensate sample.

References

1. Cundiff, R. H., Sensabaugh, A. J., and Markunas, P. C., Tobacco Sci. 6, 25 (1962).
2. Cundiff, R. H., and Markunas, P. C., Analytical Chemistry 30, 1450 (1958).

Procedure C-8 (Preliminary)

pH of Smoke Condensate

Apparatus

1. A plastic glove bag, Model X-17-17 available from I²R Corp., Cheltenham, Penn., or equivalent.
2. A magnetic-stirring motor, and an 1/8-in. dia. x 5/8-in. long Teflon-coated stirring bar.
3. A Beckman Expandomatic pH meter or equivalent.
4. A Beckman combination-electrode No. 39183.
5. Three 10 ml ground glass, stoppered, graduated cylinders.
6. Four 10 ml glass beakers.
7. A 250 ml glass wash bottle with $\frac{3}{8}$ 2-way inner joint and a separate stopper.
8. A 250 ml plastic wash bottle.
9. Tissue paper.

Reagents

1. A cylinder of nitrogen or argon fitted with a pressure regulator.
2. Freshly boiled distilled water.
3. pH 6.0 buffer solution.

Procedure

At least three 100 mg samples of the acetone-water suspensions of a condensate are to be analyzed. These samples are contained in 3.5 milliliter capacity diaphragm capped vials. The samples are washed into beakers with precisely known volumes of CO₂-free water and their pH determined.

Analysis of Sample

1. Boil ~ 200 ml of distilled water in the glass, wash bottle for ~ 10 minutes. Sparge with a stream of nitrogen or argon until the water reaches room temperature. Stopper the wash bottle and transfer to the glove bag.
2. Connect the combination electrode and stirring motor to the meter, and power supply, respectively through the service hole of the glove bag.

3. Transfer the sampler, beakers, graduated cylinders, plastic wash bottle, stirring bar, and tissue paper into the glove bag.
4. Connect the gas cylinder (via rubber tubing) to the service hole of the glove bag. Flush the bag with the gas for ~ 15 minutes.
5. Seal the bag and influte to the desired pressure.
6. Fill the plastic wash bottle with the CO₂-free water.
7. Standardize the pH meter with pH 6.0 buffer. Measure the pH of the CO₂-free water to insure the absence of acidic or basic contamination.
8. Wash the condensate samples into 10 ml beakers using just enough CO₂-free water to yield solutions containing 10 mg/ml of dry condensate.
9. Stir and measure the pH of each solution.

Procedure G-1 (Preliminary)

Operation, Adjustment, and Standardization
of the Phipps and Bird Smoking MachineApparatus

1. Cigarette Smoking Machine, 4-port constant volume, syringe type, automated, Phipps and Bird, Inc., Richmond, Virginia ⁽¹⁾. The machine is described by R. M. Wiley and J. A. Ferri, The Design of an Automated Syringe-Type Smoking Machine. Tobacco, Vol. 165 No 16, pp. 30-33, Oct. 20, 1967.
2.
 - a. 100 ml burette with 0.2 ml graduations.
 - b. 12-ft of gum rubber tubing, 3/8-in. I.D. and 3/16-in. wall.
 - c. 250 ml plastic wash bottle.
 - d. 8 mm glass or plastic "Y" about two inches long.
 - e. Pinch clamp for the gum rubber tubing.
 - f. 24-in. ring stand and clamp for burette.
 - g. Soap bubbles, the soap bubble solution may be obtained from the dime store.
3. Gas trap ⁽²⁾ consisting of an 800 ml Kjeldahl flask with a standard taper 34/45 male glass joint at the bottom that will accommodate a 50 ml glass trap with S.T. 34/45 female joint at the top. The trap is 150 mm tall by 30 mm in diameter at the bottom.

Operation, Adjustment, and Standardization of the Smoking Machine

1. An instruction pamphlet is included with the instrument. Refer to this manual for set-up, lay-out, and information on the instrument.
2. The hood on the instrument was connected to an exhaust system which provided an air flow of 210 cu. ft per min. This should be kept constant.
3. Set the puff duration at 2.0 ± 0.2 sec by adjusting the speed control knob on the front of the drive panel. One complete cycle of the drive control shaft should take 4.0 ± 0.4 sec since a puff is drawn only during the first half of the cycle.

4. Set the pulse generator on the back of the electronic timer console to give 10 register counts on the counters in exactly 2.0 sec. Locate the pulse generator behind the power supply unit in the timer console. To increase the pulse count, turn the pointer knob in a counter-clockwise direction. To decrease the pulse count, turn the pointer knob in a clockwise direction. Don't move the pointer knob more than 1/32-in. at a time.
5. Turn main power switch to on.
6. Turn timer power switch to on.
7. Set Norm-Volume check switch to volume check.
8. Set the clock Automatic-Manual switch to manual.
9. Invert a 100 ml burette and place it securely in a ring stand clamp.
10. Attach a rubber tube to the top end of the burette and run the other end to a glass "Y". To one leg of the "Y" attach a 250 ml wash bottle by means of rubber tubing and place a spring pinch clamp on the rubber tubing. From the other leg of the "Y" run about 6-ft of rubber tubing to the assembled filter holder at port No. 1. Remove the rubber membrane on the filter holder, and attach the rubber tubing in its place.
11. Pour about 125 ml of soap bubble solution into a small beaker.
12. Raise the soap solution over the open end of the burette and by means of the plastic squeeze bottle, draw about 80 ml of liquid up the burette to wet the burette. Allow the excess liquid to drain from the burette.
13. Change the pinch clamp from the rubber tubing going to port No. 1 to the rubber tubing going to the plastic squeeze bottle.
14. Place a soap bubble on the bottom open end of the burette.
15. Press the manual control push button on the cord extending from the filamatic control unit to initiate one puff cycle. This should cause a displacement of the soap membrane, record its position and put another soap membrane on the burette. Repeat the operation and record the new position of the first membrane. The volume difference is equal to the puff volume. Repeat the

membrane displacement until reproducibility is obtained. This usually requires four or five volume readings. The volume of each port should be 35.0 ± 0.5 ml. Important. Check all four port volumes before making any volume adjustments. If the ports have different volumes, then an individual port volume adjustment will have to be made. If all four port volumes are about the same, but not within the desired limits, then a gross volume adjustment of all four ports should be made first.

16. Single port volume adjustment. Loosen the set screw on the eccentric cam located next to the base of the syringe on the lower shaft. Move the cam up or down as required and lock the set screw. Measure the puff volume again as described previously.
17. Gross volume, 4-port adjustment. Cut off the main power. Place the metal blocks, located in the holder on the right hand side of the machine, to the rear of each drive shaft so that when the shaft is manually turned $1/4$ -turn clockwise, the body of the micrometers will rest on the blocks. Insert a tool into the hole at the center of the drive shaft and push in a clockwise direction to loosen the shaft tension. Turn the shaft clockwise to the $3/4$ turn position and place the blocks in front of the shaft. Seat the micrometer bodies firmly on the blocks. Adjust each micrometer to obtain more or less volume as needed. Trial and error is used in this adjustment. Place the tool in the hole at the center of the shaft and push counter clockwise to tighten the shaft. Remove the blocks before turning the main power switch on at which time the drive shaft automatically returns to the starting position. Measure the puff volume again and repeat the adjustments until the puff volume falls within tolerance.
18. The syringes should contain about one-fourth inch of Hi-vacuum oil. Unscrew the metal head of the syringe and tilt slightly upward in order to add oil. Avoid letting the oil run through the delivery end of the syringe.

Smoking

1. Pre-select and weigh each conditioned cigarette and place it in a tray in the assigned order it is to be smoked.
2. Insert the leak checked, completely assembled, pre-weighed, and numbered filter holders into their respective numbered port receivers.
3. Pull the thread past the last holding post on the opposite side of the cabinet. Secure the thread by sliding it through the spring coils. Pass the thread over the micro-switches, pressing them down and secure the thread to the next spring, continue on to the last coil spring.
4. Mount the cigarettes by inserting them through the holding bar holes into the rubber membrane ports of the filter assembly. The thread should rest on the top of each cigarette at the 23 mm butt length mark. If the micro-switches are properly set, all lights on the time console unit should be out.
5. Raise the lighter bar and position the lighters, turn the "one" minute timer on, and press the lighter button about 20 to 30 sec before the puff starts to allow the lighters to get hot. Move the hot lighters against the cigarettes about 3 to 5 sec before the puff starts, and hold the lighters there until the first puff is drawn.
6. Lower the lighters and re-position the cigarettes if necessary.
7. Record any misbehavior or unusual characteristics while the cigarettes are smoked down to the 23 mm butt.
8. Allow the cigarette to remain one minute longer after burning through the thread. This prevents loss of smoke from the last puff when the butt is removed.
9. Mount the second cigarette of each set and repeat the smoking operation until four cigarettes have been smoked at each port on each filter.
10. Remove each complete filter assembly and weigh immediately on an analytical balance to the nearest 0.1 mg.
11. Dissassemble the filter holder, carefully remove the filter and push it into a pre-dried 28.0 ml vial and cap it with a Teflon-lined rubber diaphragm and screw cap for hypodermic syringe removal of sample.

12. Immediately add 20.0 ml of dioxane containing 0.3% isopropanol or any other specified reagent and recap the sample container immediately. From this point further analysis can be carried out on the sample.

References

1. Ogg, C. L., J.A.O.A.C., Vol. 47 No 2, pp. 356-358, 1964.
2. R. J. Reynolds Tobacco Company, Research Department Analytical Method No. SM-T-1.

Procedure G-2 (Preliminary)

The Operation of The Griffith Still

Apparatus

1. Griffith steam distillation apparatus, available from Lab Glass, Inc., Kingsport, Tennessee.
2. Six, 250 ml wide mouth, flat bottom volumetric flasks.
3. Polyethylene coated (1-in. x 1/2-in.) magnet and a magnetic stirring motor.

Reagents

1. 36+ $\%$ reagent grade hydrochloric acid to prepare a 0.2 N solution and a 1 + 9 (hydrochloric acid + H₂O) solution.
2. Dissolve 300 g/l NaOH and saturate with NaCl. After 24 hours decant supernate.
3. Silicone high vacuum grease from Dow Corning Corporation, Midland, Michigan.

Description of Apparatus

The pieces of equipment that make up the Griffith steam distillation apparatus are described in detail⁽¹⁾. Basically, the unit consists of a bank of six stills mounted in an enclosed wooden case open across the front. (Inside of the case is a master electrical line which is plugged into an electrical source to give power to a light and a transformer variac tied into the main heater switch used for activating the glass coil heaters wrapped around each still pot.) The steam is routed through a manifold provided with a steam pressure gauge and six outlets. This manifold is connected at one end to a steam source and at the other end to a condensate return line. Individual needle valves regulate the flow of steam to the still pot (through a stopcock). Other manifold systems are built in for providing water to and from the condensers and for the connection of waste outlets from the stills via the water aspirator pump to the drain.

The separate still units comprising the steam distillation apparatus (as received) are easily assembled. Lubricate the ball joints, the

respective Teflon stopcocks and ground glass Pyrex stoppers with high vacuum grease. When the steam and water inlet lines, the electrical plug line, and the drain lines are connected to their respective sources and exits, the apparatus is considered ready for operation.

Operation

1. Insure that the heater switch is off and turn on the master power.
2. Turn on the water valve which cools the condensers and operates the aspirator drain pump.
3. Turn on the steam valve. After the condensed water in the steam line has been eliminated through the condensate return line, the pressure is regulated to ~ 15 lbs with the valve by referring to the pressure gauge. (The 3-way stopcocks to the still pots are in the OFF position.)

Note:

Before setting out to use the apparatus for the first time, the needle valves to the individual units below the 3-way Teflon stopcocks need to be adjusted so that the rates of distillation at each port are approximately the same. Suggested distillate collection rate is ~ 12 ml/min. The main heater switch should be "ON" and the variac set at ~ 100 v to avoid volume build-up in the still pots during this operation.

4. Expel any condensate in the line between the needle valves and the 3-way stopcocks into the still pots. Individually drain each still by turning the respective stopcock 180 degrees. Be sure after the still is drained to shut off the stopcock before proceeding to turn another cock to drain the next still.
5. With all stopcocks turned "Off" remove the stopper from a still pot. Pipette 5 ml of 0.2 N HCl into the still pot and then add the size aliquot of sample to be distilled. Replace the stopper and proceed in the same manner until the desired number of still pots are loaded with samples.

Note:

For the first distillation run of the day, it is desirable to add an aliquot of a standard nicotine and/or phenol solution to one of the still pots.

6. Place an appropriate collection vessel under the U tube receiver then turn the main heater switch "On". Set the variac at ~ 100v and allow 5 min for the contents to heat.
7. In sequence, carefully open the 3-way Teflon cocks one at a time to admit steam into the still pots. Even if a still pot has no sample or standard it is desirable to run steam.
8. After ~ 150 ml of distillate has been collected, turn off the heater switch and the Teflon stopcocks. Remove the collection vessels from under the U tube receivers.
9. If only the total alkaloids (as nicotine) is to be determined, discard the first distillate. If the phenol fraction is also desired, save this cut and proceed by placing a 250 ml, wide mouth, flat bottom volumetric flask containing 10 ml of 1 + 9 (hydrochloric acid + water) under each U tube receiver.

Note:

For best recovery attach a 1/4-in. piece of Teflon tubing to the end of each U tube receiver. Allow the tip of the tube to extend into the acid during the total alkaloid distillation.

10. Remove the still stoppers one at a time and drain in 5 ml of the 30% NaOH-sat.-NaCl solution into each pot from the measuring dispenser tubes mounted above the still pots.
11. Replace the respective still stoppers and turn the heater switch to the "On" position. Slowly open the stopcocks in sequence to the individual still pots so as to avoid a sudden surge of alkaline liquid into the condenser unit, etc.
12. After ~ 240 ml of distillate is collected in volumetric flask, (marked at 260 ml volume) the Teflon cock is shut off. When all the distillations are completed, turn the heater switch to the "Off" position. Carefully remove the distillate receiver flasks one at a time and wash down the Teflon extension tube with distilled water. Then adjust the volume of the distillate(s) to the 260 ml mark with distilled water.
13. Place a polyethylene coated metal stirring bar into one of the flasks and equilibrate for ~ 1 min with a magnetic stirring

motor. Remove the bar, wash it with distilled water and repeat the procedure until all the solutions are equilibrated. Then proceed with the nicotine analysis. ⁽¹⁾

Note:

If the first distillate was saved, adjust it to a known volume with distilled water and mix, etc., before proceeding with the phenol determination. ⁽²⁾

Clean Up

1. Drain the individual still pots in the prescribed manner.
2. Rinse the stoppers and still pots with about 15 ml of 0.5 N HCl solution. Drain the stills and close the stopcocks.
3. Open the stopcock to one still. Set a beaker containing about 50 ml of 0.5 N HCl under the tip of the Teflon receiver tube. Pinch the rubber tube on the receiver side arm closed. As this is done, the acid is pulled up into the condenser, down into the still and out the drain.
4. Repeat with a distilled water wash so as to leave no acid in the metal condenser. Shut off the Teflon cock and open the next one to the drain and continue the clean-out procedure until all the dirty stills are cleaned.
5. Turn off the steam valve.
6. Wait 5 min and turn off the water valve.
7. Then turn the variac control to (0) zero volts and turn the master heater switch to the "Off" position.

References

1. Griffith, R. B., Tobacco Sci. 1, 130-137 (1957).
2. Oakley, Elizabeth, T., Millham, J.O. and Weissbecker, L., Anal. Chem. Acta. 31, 272 (1964).

10.00 APPENDIX B EXPERIMENTAL RESULTS

This section is a partial reproduction of the results submitted to the project officer on 8-24-70.

Forms like that on page 181 were completed for experimental cigarettes numbers 2 to 23, and forwarded to the project officer. Tables 3-1, 3-2, 3-3, 3-12, 3-13, 3-14, 3-15, 3-16, 3-17 and 3-18 were also part of the submission of results. A cover letter summarizing observations which later were incorporated into the text of Section 3.00 of this progress report was also included. These portions of the results submission are omitted here to avoid duplication.

To: G. B. Gori, Project Officer
From: Analytical Chemistry Division, Oak Ridge National Laboratory
Subject: Analytical Results Under NIH-AEC Agreement No. 40-117-67

Results of the chemical analysis of experimental cigarettes numbers 1 to 23 for TOTAL PARTICULATE MATTER, WATER, NICOTINE, TAR, ACETALDEHYDE, and ACROLEIN are appended. Results of the analysis of particulate matter reported on 6-29-70 correspond to those generated using standard smoking parameters and the smoking machine manufacturers recommended hood velocity of approximately 300 cubic feet per minute. Results of the analysis of particulate matter reported here correspond to those generated using standard smoking parameters and a hood velocity adjusted such that the Kentucky Reference Cigarette yielded $11.1 \pm .2$ puffs per cigarette.

DEFINITION OF TERMS

1. TPM - "Total Particulate Matter", the gross increase in weight of the Cambridge Filter Assembly attending the smoking process; includes the weights of water and nicotine.
2. Water - The water content of the TPM as determined using gas chromatography.
3. Nicotine - The alkaloid content (expressed as nicotine) of the TPM as determined using the Griffith Still and ultraviolet spectrophotometry.
4. "Tar" - The weight of the TPM minus the combined weights of nicotine and water.
5. Acetaldehyde - The acetaldehyde content of the material that passes through the Cambridge filter. No known interference.
6. Acrolein - The acrolein content of the material that passes through the Cambridge filter. The values reported are known to contain a minor (insignificant considering experimental error) contribution from methyl acetate.
7. mg/cig - $\mu\text{g/cig}$ - Results expressed on a milligrams or micrograms per cigarette basis. For the analyses of particulate matter, the values found per filter pad were divided by four (four cigarettes per pad) and the values from three or four filter pads were averaged. For the analyses of the gas phase, the values

per filter pad were divided by one (one cigarette per pad), and the values from three filter pads were averaged.

8. mg/g tob, $\mu\text{g/g}$ tob. - Results expressed on a milligrams or micrograms per gram of tobacco burned. For the analyses of particulate matter and gas phase, the weights of the cigarettes sampled were multiplied by 0.729 ($[85 \text{ mm} - 23 \text{ mm}] \div 85 \text{ mm}$) to obtain a measure of the weight of tobacco burned. The quantity of the component measured was then divided by this weight "burned".
9. Wt. % TPM - Results of water, nicotine, and tar determinations expressed as the weight percent of the Total Particulate Matter.
10. $\mu\text{g/l}$ smoke - Results expressed as micrograms per liter of smoke generated. Results of the gas phase analyses were first calculated on a micrograms per puff basis. The values in micrograms per puff were divided by 0.035 liters per puff to convert to micrograms per liter. No corrections were made for ambient temperature or atmospheric pressure.
11. S - Standard deviation for the small samplings (3-4 determinations). All results are reported as the mean of at least three determinations. The precision of the determinations are given in terms of the standard deviation. Standard deviations were calculated from the range using the expression $S = R/C$ where S = standard deviation, R = range, and $C = 1.91$ for 3 values and $C = 2.24$ for 4 values (E. L. Bauer, A Statistical Manual For Chemists, Academic Press, New York, N. Y., 1960). Results for which the relative standard deviation (standard deviation $\div$ average) is 5% of the average are generally accepted. For the determinations of water, acetaldehyde and acrolein, relative standard deviations of 10% are generally accepted.

DEFINITION OF RESULTS

Four ports (16 cigarettes) were used for each analysis of particulate matter. Data from one of the four ports was neglected only if it failed the statistical Q-test and/or there was reason to suspect a systematic error, e.g. leakage. In those cases where results were considered

questionable, a new series of 16 cigarettes was sampled. Results presented here correspond to the data generated with best precision over the four ports. In those cases where more than one complete series of cigarettes was sampled, and we could find no justification for omitting the results from any given series, the results reported correspond to the average of the results obtained from all the series examined.

Three cigarettes were sampled in analyses of the gas phase (new filters for each cigarette). Additional cigarettes were sampled whenever the data generated from the triplicate analyses were of poor precision or a systematic error was suspected. Results reported here correspond to the data generated with best precision over three single-cigarette analyses.

Relative standard deviations for the six analyses expressed in the given units are summarized below.

Average Relative Standard Deviation
Percent of Average

	<u>TPM</u>	<u>Water</u>	<u>Nicotine</u>	<u>Tar</u>	<u>Acetaldehyde</u>	<u>Acrolein</u>
mg/cig	2.30	6.00	2.99	2.40		
µg/cig					2.51	4.15
mg/g tob	2.33	6.33	2.91	2.40		
µg/g tob					2.57	4.10
Wt. % TPM		5.15	2.43	0.89		
µg/l smoke					2.64	3.94

Sample Characteristics

Code	<u>Average Weight of Cigarette (Gm)</u>		<u>Puffs to 23 mm Butt</u>	
	<u>PM Analysis</u>	<u>Gas Phase Analysis</u>	<u>PM Analysis</u>	<u>Gas Phase Analysis</u>
1	1.135	1.120	11.2	11.0
2	1.223	1.231	10.7	11.2
3	1.240	1.228	10.5	10.1
4	1.216	1.207	11.7	11.8
5	1.225	1.203	9.9	9.5
6	1.214	1.173	11.3	11.0
7	1.220	1.163	9.9	9.1
8	1.254	1.178	12.2	11.7
9	1.226	1.196	9.0	8.8
10	1.152	1.115	10.9	11.1
11	1.154	1.114	11.5	12.0
12	1.097	1.043	9.1	9.3
13	1.341	1.209	8.9	8.5
14	0.981	0.992	6.0	6.0
15	1.221	1.167	7.0	7.0
16	1.188	1.160	8.2	8.0
17	1.062	1.039	7.0	6.5
18	0.964	0.981	6.8	7.0
19	1.072	1.078	7.0	7.0
20	1.376	1.304	10.2	11.0
21	0.834	0.807	6.0	5.9
22	1.391	1.411	9.4	9.0
23	1.308	1.214	8.8	8.0

SUMMARIZED RESULTS

Reporting Date: 8-24-70

Last Report: 6-29-70

Date Analyzed: July-August 1970

Samples: Experimental Cigarettes, 1-23

Units: Milligrams per cigarette

Standard Deviation

Sample	TPM		Water		Nicotine		Tar	
1	44.6	1.17	5.2	0.42	2.74	0.02	36.6	0.74
2	35.4	0.84	3.7	0.18	2.11	0.07	29.6	0.67
3	29.5	0.54	2.8	0.08	1.94	0.05	24.8	0.53
4	44.2	0.56	5.2	0.32	2.92	0.05	36.2	1.06
5	32.4	0.61	3.6	0.25	1.86	0.06	27.0	0.54
6	36.4	0.48	4.5	0.02	2.26	0.07	29.6	0.41
7	31.9	0.68	3.5	0.07	2.01	0.04	26.4	0.56
8	44.3	1.32	5.8	0.44	2.62	0.12	35.9	0.76
9	28.1	0.59	2.9	0.45	1.57	0.03	23.6	0.54
10	41.6	1.17	5.3	0.65	2.98	0.10	33.3	0.54
11	46.1	1.57	5.2	0.35	3.09	0.03	37.8	1.19
12	33.4	0.57	4.2	0.20	3.22	0.06	26.0	0.62
13	16.6	0.21	2.3	0.07	0.34	0.02	13.9	0.18
14	16.4	0.50	2.9	0.19	0.21	0.01	13.4	0.34
15	19.7	0.67	3.1	0.27	0.76	0.02	15.9	0.42
16	20.0	0.19	2.7	0.04	0.92	0.02	16.4	0.25
17	15.9	0.40	2.7	0.15	0.76	0.04	12.5	0.40
18	19.2	0.40	3.5	0.33	0.82	0.02	14.9	0.35
19	20.9	0.36	3.7	0.07	0.97	0.03	16.3	0.39
20	37.9	0.95	2.9	0.08	2.02	0.05	33.0	0.93
21	7.0	0.10	0.7	0.09	0.22	0.01	6.1	0.09
22	33.4	1.33	3.3	0.06	1.47	0.06	28.6	1.26
23	35.2	1.34	4.2	0.17	1.42	0.05	29.7	1.23

SUMMARIZED RESULTS

Reporting Date: 8-24-70

Last Report: 6-29-70

Date Analyzed: July-August 1970

Samples: Experimental Cigarettes, 1-23

Units: Milligrams per gram tobacco burned

Standard Deviation

Sample	TPM		Water		Nicotine		Tar	
1	55.4	1.35	6.5	0.52	3.40	0.01	45.5	0.86
2	40.3	1.01	4.2	0.21	2.39	0.09	33.6	0.81
3	32.5	0.59	3.0	0.08	2.13	0.06	27.3	0.58
4	50.4	0.68	6.0	0.37	3.34	0.06	41.1	0.66
5	36.8	0.75	4.0	0.30	2.10	0.08	30.7	0.63
6	41.6	0.54	4.2	0.03	2.58	0.06	34.0	0.46
7	36.2	0.78	4.0	0.06	2.28	0.05	30.1	0.66
8	49.7	1.46	6.5	0.49	2.94	0.13	40.3	0.84
9	32.5	0.58	3.4	0.51	1.80	0.03	27.1	0.72
10	50.9	1.38	6.5	0.79	3.64	0.12	40.7	0.67
11	56.7	1.84	6.4	0.42	3.80	0.04	46.5	1.38
12	43.0	0.61	5.4	0.27	4.14	0.08	33.5	0.70
13	17.3	0.23	2.4	0.07	0.35	0.01	14.6	0.20
14	23.4	0.80	4.1	0.28	0.31	0.01	19.0	0.54
15	22.9	0.78	3.6	0.31	0.89	0.03	18.4	0.49
16	23.7	0.30	3.4	0.36	1.09	0.02	19.4	0.30
17	21.2	0.58	3.6	0.19	1.01	0.05	16.6	0.64
18	28.2	0.59	5.1	0.50	1.21	0.03	21.9	0.48
19	27.6	0.50	4.8	0.04	1.28	0.04	21.4	0.50
20	38.6	0.96	3.0	0.08	2.06	0.06	33.6	0.95
21	11.8	0.16	1.2	0.15	0.37	0.02	10.3	0.16
22	33.6	1.33	3.3	0.06	1.48	0.06	28.8	1.27
23	37.8	1.48	4.5	0.18	1.52	0.05	31.8	1.38

SUMMARIZED RESULTS

Reporting Date: 8-24-70

Last Report: 6-29-70

Date Analyzed: July-August 1970

Samples: Experimental Cigarettes, 1-23

Units: Weight percent of TPM

Standard Deviation

Sample	TPM		Water		Nicotine		Tar	
1			11.67	0.64	6.15	0.15	82.18	0.49
2			10.42	0.44	5.94	0.10	83.65	0.39
3			9.39	0.29	6.57	0.11	84.05	0.39
4			11.86	0.72	6.63	0.04	81.80	1.14
5			10.86	0.80	5.72	0.12	83.41	0.80
6			12.37	0.11	6.21	0.08	81.42	0.12
7			11.00	0.13	6.28	0.06	82.71	0.14
8			13.11	0.61	5.92	0.11	80.98	0.70
9			10.40	1.46	5.57	0.17	84.04	1.36
10			12.76	1.32	7.15	0.21	80.09	1.39
11			11.35	0.39	6.71	0.16	81.95	0.22
12			12.47	0.68	9.63	0.31	77.91	0.87
13			14.00	0.28	2.04	0.11	83.96	0.36
14			17.85	0.56	1.31	0.05	81.34	0.83
15			15.32	0.33	3.87	0.24	80.50	0.60
16			13.62	0.45	4.59	0.07	81.79	0.50
17			16.83	0.92	4.78	0.16	78.39	0.78
18			18.02	1.58	4.28	0.09	77.70	1.53
19			17.60	0.56	4.63	0.08	77.77	0.54
20			7.67	0.32	5.32	0.15	87.02	0.45
21			9.91	1.25	3.15	0.20	86.94	1.18
22			9.84	0.40	4.41	0.06	85.75	0.40
23			11.80	0.38	4.02	0.04	84.18	0.38

SUMMARIZED RESULTS

Reporting Date: 8-24-70

Last Report: 6-29-70

Date Analyzed: May-June 1970

Samples: Experimental Cigarettes, 1-23

Units: Micrograms per cigarette

Standard Deviation

Sample	Acetaldehyde		Acrolein		CO		CO ₂	
1	1213	7.3	58.1	2.2				
2	1280	36.2	57.9	3.1				
3	1044	15.2	46.9	0.7				
4	1424	24.1	71.6	2.2				
5	1169	20.4	63.0	2.2				
6	1191	20.4	50.2	1.5				
7	1006	46.6	39.8	0.6				
8	1112	22.0	54.3	2.5				
9	783	39.8	34.1	1.8				
10	1017	30.9	42.2	1.9				
11	1113	18.9	58.9	2.7				
12	805	40.9	29.3	1.3				
13	1380	34.6	62.4	2.9				
14	980	9.4	52.2	0.8				
15	996	39.8	35.5	2.0				
16	1171	45.6	59.5	3.2				
17	900	7.3	48.0	2.2				
18	1005	27.8	55.1	3.5				
19	951	26.2	46.4	1.6				
20	1373	33.5	45.2	2.2				
21	675	21.5	31.9	1.2				
22	1493	15.2	68.3	3.1				
23	1338	22.0	59.5	3.3				

SUMMARIZED RESULTS

Reporting Date: 8-24-70

Last Report: 6-29-70

Date Analyzed: May-June 1970

Samples: Experimental Cigarettes, 1-23

Units: Microgram per gram tobacco burned

Standard Deviation

Sample	Acetaldehyde		Acrolein		CO		CO ₂	
1	1483	10.5	71.3	2.9				
2	1424	30.4	64.4	4.2				
3	1168	22.5	52.3	0.5				
4	1619	32.5	81.3	2.5				
5	1334	32.5	71.9	2.6				
6	1392	24.6	58.7	1.8				
7	1186	50.3	47.0	0.8				
8	1295	26.7	63.2	2.9				
9	898	55.0	39.0	2.4				
10	1251	30.9	51.9	1.9				
11	1370	23.6	72.6	3.4				
12	1059	54.5	38.5	1.6				
13	1563	33.5	70.7	3.1				
14	1353	25.7	72.1	0.7				
15	1169	44.0	41.8	2.5				
16	1382	55.0	70.2	3.9				
17	1187	7.9	63.3	3.1				
18	1403	47.7	76.9	5.1				
19	1210	34.1	58.9	2.1				
20	1441	38.2	47.5	2.2				
21	1144	35.1	54.0	2.0				
22	1450	15.2	66.3	1.7				
23	1509	14.1	67.2	3.6				

SUMMARIZED RESULTS

Reporting Date: 8-24-70

Last Report: 6-29-70

Date Analyzed: May-June 1970

Samples: Experimental Cigarettes, 1-23

Units: Micrograms per liter of smoke

Standard Deviation

Sample	Acetaldehyde		Acrolein		CO		CO ₂	
1	3104	52.4	148.8	9.1				
2	3301	131.4	149.3	8.2				
3	2960	80.6	132.7	1.9				
4	3466	136.1	174.0	6.9				
5	3516	79.6	189.6	6.4				
6	3117	92.7	131.3	5.4				
7	3165	147.6	125.3	3.8				
8	2685	30.4	131.2	6.0				
9	2534	53.9	110.2	2.4				
10	2622	62.8	108.8	3.6				
11	2650	45.0	140.2	6.5				
12	2466	66.0	89.7	1.5				
13	4689	149.2	211.8	2.1				
14	4666	45.0	248.7	3.7				
15	4064	161.8	145.0	8.2				
16	4181	161.8	212.4	11.5				
17	3675	29.8	195.9	9.1				
18	4102	113.6	224.8	14.0				
19	3883	106.3	189.2	6.4				
20	3565	86.9	117.5	5.7				
21	3309	167.5	156.3	6.1				
22	4740	49.2	216.8	9.8				
23	4779	79.6	212.6	11.8				

COMPLETED ANALYSES

Reporting Date: 8-24-70

Last Report: 6-29-70

Date Analyzed: 7/70 - 8/70

Sample: 1

1. Particulate Matter

	<u>mg/cig.</u>	<u>S</u>	<u>mg/g. tob</u>	<u>S</u>	<u>Wt% TPM</u>	<u>S</u>
1. TPM	44.6	1.17	55.4	1.35	----	----
2. Water	5.2	0.42	6.5	0.52	11.17	0.64
3. Nicotine	2.74	0.02	3.40	0.01	6.15	0.15
4. "Tar"	36.6	0.74	45.5	0.86	82.18	0.49

11. Gas Phase

	<u>ug/cig.</u>	<u>S</u>	<u>ug/g tob.</u>	<u>S</u>	<u>ug/l smoke</u>	<u>S</u>
1. CO	_____	_____	_____	_____	_____	_____
2. CO ₂	_____	_____	_____	_____	_____	_____
3. NO ₂	_____	_____	_____	_____	_____	_____
4. Acetaldehyde	<u>1213</u>	<u>7.3</u>	<u>1483</u>	<u>10.5</u>	<u>3104</u>	<u>52.4</u>
5. Acrolein	<u>58.1</u>	<u>2.2</u>	<u>71.3</u>	<u>2.9</u>	<u>148.8</u>	<u>9.1</u>
6. Formaldehyde	_____	_____	_____	_____	_____	_____
7. HCN	_____	_____	_____	_____	_____	_____
8. pH (average, as defined)	_____	_____	_____	_____	_____	_____

Puff #1 _____
 #2 _____
 #3 _____
 #4 _____
 #5 _____
 #6 _____
 #7 _____
 #8 _____
 #9 _____
 #10 _____
 #11 _____

11.00 Operating Protocol - Appendix C

The ORNL Protocol defines the policy under which this work is carried out and is a complete summary of the procedures in use and to be used for the analyses that are this facility's responsibility. We assume that the intentions and procedures outlined in the protocol have the approval of the Tobacco Working Group. Major changes can only be made with the approval of the project officer.

The final protocol will largely be a collection of completely detailed analytical procedures actually used for the analyses of the experimental cigarettes. As such a document cannot be prepared until every analysis has been successfully carried out, this protocol must be considered preliminary. It is our intention that shortcomings of the procedures selected and potential alternative procedures be suggested by the program coordinators and by other contractors.

PROTOCOL — Oak Ridge National Laboratory

Addendum to
AEC-NIH Agreement 40-117-67

- I. Introduction
- II. Anticipated Work Load
- III. Sampling
 - A. Conditioning and Smoking
 - B. Collection
 - 1. TPM and "tar"
 - 2. Gas Phase
 - a. CO, CO₂
 - b. NO, NO₂
 - c. Acetaldehyde, Acrolein
 - d. Formaldehyde
 - e. HCN
 - f. pH
 - 3. Fresh Condensate
 - C. Sample Size Requirements
- IV. Analytical Procedures
 - A. TPM and "tar"
 - B. Gas Phase
 - 1. CO, CO₂
 - 2. NO, NO₂
 - 3. Acetaldehyde, Acrolein
 - 4. Formaldehyde
 - 5. HCN
 - 6. pH
 - C. Condensate
 - 1. Water and Acetone
 - 2. Nicotine
 - 3. "tar"
 - 4. Phenol, o-cresol, m + p-cresol
 - 5. pH
 - 6. Weak Acids and Very Weak Acids

7. Phenanthrene, Benz(a)anthracene, Benzo(a)pyrene

8. Dibenz(a,j)acridine

V. Network of Operations and Work Schedule

VI. Reporting Results

VII. References

Appendix A, Alternate Procedures

Appendix B, Possible Added Responsibilities

Appendix C, Report Format and Forms

I. Introduction

We propose to function as the site responsible for the chemical analyses included in the National Cancer Institute program for elucidating the characteristics of a series of experimental cigarettes. This responsibility will temporarily supplant general research as the primary activity of our effort.

The purpose of this protocol is to define the policies and procedures that constitute this effort. Each procedure must be successfully applied to the analysis of the experimental cigarettes before a completely detailed, final, protocol can be prepared. This document must be viewed as a presentation of intended operations and, as such, is preliminary in nature. The final protocol will evolve as this effort progresses.

The governing policy of this effort is that the Tobacco Working Group is the final authority in selecting compounds to be monitored and analytical procedures. Initially considered analytical procedures must be selected from the procedures suggested by the Tobacco Working Group. We have the right to modify basic procedures such as to make them more workable in our hands and to consider alternate procedures which we feel are superior to suggested procedures. New or significantly modified procedures will be applied to the analyses of samples only after approval of the procedures by the project officer. New procedures and significant modifications of basic procedures now under consideration are summarized in Appendix A.

New problem areas may require attention as this effort progresses. A summary of potential new problem areas is given in Appendix B.

II. Anticipated Work Load

It is anticipated that at least 46 samples of smoke condensate produced by routine large scale methods will each require analysis for: water, alkaloids, tars, acetone, phenol, o-cresol, m + p cresol, nicotine, nornicotine, pH, weak and very weak acids, phenanthrene, benz(a)anthracene, and benzo(a)pyrene. Analyses for dibenz(a,j)acridine may be included later. In addition, cigarettes corresponding to those which generate the condensate will be forwarded for analyses of total particulate matter, gas phase components (carbon monoxide, carbon dioxide, oxides of nitrogen, acetaldehyde, acrolein, formaldehyde, hydrogen cyanide, and pH) and freshly prepared condensate (the same components as for the large scale production condensate).

III. Sampling

A. Conditioning and Smoking

Cigarettes will be weight selected to within ± 20 mg of the average weight of at least 50 cigarettes after at least 24 hours of equilibration at $75 \pm 2^{\circ}\text{F}$ and $60 \pm 2\%$ R.H. Cigarettes will be smoked in the same room used for their equilibration, that is, at $75 \pm 2^{\circ}\text{F}$ and $60 \pm 2\%$ R.H. Each smoking machine will be adjusted to draw a 35 cc puff, as measured at the port of the filter holder, of 2 second duration once every minute. Smoking will be stopped when a 23 mm butt length is reached.

Samples for the determination of "tar" and nicotine alkaloids will be generated using a four-port version (manufactured by Phipps and Bird, Inc., Richmond, Va.) of the Philip Morris Cigarette 20-channel machine. Using separate cigarettes and appropriate traps, the same machine will be used to generate samples for the determination of formaldehyde and hydrogen cyanide in the gas phase of smokes.

Samples for the determination of carbon monoxide and carbon dioxide, oxides of nitrogen, and pH, will be generated using a single-channel home-constructed machine employing a Filamatic unit (National Instrument Co., Baltimore, Md.) of the type used on the Phipps and Bird Machines for the puffing mechanism. Samples for the determination of acetaldehyde and acrolein will be generated using a home-constructed machine employing Dyna-Vac pumps as puffing mechanisms.

Fresh smoke condensate will be generated using a model No. IIIa smoking machine manufactured by Heinr. Borgwaldt of Hamburg, Germany. The condensate will be generated in a room illuminated with yellow light as well as being maintained at $75 \pm 2^{\circ}\text{F}$ and $60 \pm 2\%$ R.H.

In the event samples of cigarettes and condensates cannot be analyzed immediately, the samples will be sealed in plastic bags and stored in a freezer at approximately 20°F . When ready for analysis, the samples will be thawed while in the bags, removed, and conditioned at 75°F and 60% R.H. before use.

B. Collection

1. TPM and "tar"

Samples will be collected on Cambridge Filters (Phipps and Bird, Inc., Richmond, Va.) for the determination of total particulate matter, "tar," and nicotine alkaloids. The weight of the material collected on each

filter will be determined immediately after smoking to yield values for total particulate matter. The filters will be placed in sealed containers for later determinations of water and nicotine alkaloids and, thus, "tar." Not more than four cigarettes will be smoked through any one filter.

2. Gas Phase

Additional cigarettes will be smoked onto Cambridge Filters. Samples of gas phase components will be prepared from that material which passes the filter. Not more than one cigarette will be smoked through any given filter.

As many cigarettes will be smoked as necessary to produce reliable quantitative values for the components of interest. In no case will the smoke of less than one whole cigarette be sampled.

a. Carbon monoxide, Carbon dioxide

The gas phase of a whole cigarette will be routed to a balloon trap. The technique will be comparable to that employed at R. J. Reynolds Research (4,9).

b. Oxides of Nitrogen

The gas phase of at least one whole cigarette will be contained in a flask containing the Saltzman reagent (12). See Appendix A.

c. Acetaldehyde and Acrolein

The gas phase of at least one whole cigarette will be drawn into a sample loop a puff at a time. Between puffs, the contents of loop will be directed onto the analytical gas chromatography column held at a temperature known to completely retain acetaldehyde and acrolein. The technique will be comparable to that of Laurene et al (1,8). See Appendix A.

d. Formaldehyde

The gas phase of at least one whole cigarette will be drawn through a trap containing sodium metabisulfite (9). The reagent will be contained in a trap of a design suggested by R. J. Reynolds Research (9). See Appendix A.

e. Hydrogen cyanide

The gas phase of at least one whole cigarette will be drawn through a trap containing ascarite. The contents of the trap will be recovered by washing the ascarite with water (10). See Appendix A.

f. pH

The smoke chamber described by Sensabaugh (15) will be used for the "collection" and measurement of pH.

3. Fresh Condensate

It will be necessary to generate approximately 20 g of condensate to have sufficient sample for the determination of all of the selected "indicator" compounds. Assuming that this quantity should be collected in a single day if it is to be considered fresh, and that one cigarette will generate approximately 30 mg of condensate, approximately 900 cigarettes must be smoked in one day. A new robot smoker is on hand. Smoking conditions will be the same as described above with the addition that smoking will be carried out in a yellow room. The condensate will be removed from the traps and prepared for analysis using the same procedures employed at Melpar for the large-scale condensate production.

C. Sample Size Requirements

The approximate weights of condensate and numbers of cigarettes required for each analysis is given with the descriptions of the respective analytical procedures. A summary of the estimated requirements is presented here.

Assuming a 2 g sample for the analyses of the polycyclic aromatic hydrocarbons, and three determinations per cigarette for a total of 6 g of condensate; 0.1 g each for triplicate determinations of water plus acetone, phenol + cresols, weak and very weak acids, pH, and nicotine for a total of 1.5 g; and an additional equal quantity for possible later analyses and/or in the event of determinate errors, approximately 15 g of condensate will be required. Including unavoidable waste and the possibility of later analyses for dibenz(a,j)acridine, 25 g of condensate is requested for each cigarette sample. The number of cigarettes required to produce the sample must be noted.

Assuming an average cigarette will produce 30 mg of condensate, and allowing for determinate errors, waste, the generation of gas phase samples and samples for the determination of "tar" and nicotine values, a total of 1000 cigarettes of each type is requested.

IV. Analytical Procedures

Completely detailed, step-by-step procedures will be prepared in the initial phases of the project. Initial analyses will be carried out using the general sequence of operations described in the quoted references. Promising alternative procedures (see Appendix A) will be applied in parallel with those described in the references and will supplant the

referenced procedures only after approval of the project officer. Following is a general description of and reference to initial applied procedures.

A. TPM and "Tar" (Minimum of 16 cigarettes)

After determining the weight of wet tar, the Cambridge pad plus wipings from the holder will be suspended in dry dioxane-isopropanol and its water content determined by gas chromatography on Porapak Q. The suspension will be transferred to a Griffith still and steam distilled according to Ogg (14). The distillate will be analyzed spectrophotometrically for nicotine (14) and "tar" determined by subtracting the weights of nicotine and water from the weight of total particulate matter. See Ogg (14) and Schultz and Spears (17).

B. Gas Phase Components

1. Carbon monoxide and carbon dioxide (Minimum of 3 cigarettes).

The contents of the balloon-trap will be sampled either by gas-tight syringe or by exhausting the contents through a calibrated gas sampling loop. The sample will be analyzed gas chromatographically (4).

2. Oxides of Nitrogen (Minimum of 3 cigarettes)

The contents of the trap will be allowed to stand for at least one hour for the air oxidation of NO to NO₂. The NO₂ will be determined colorimetrically (12). See Appendix A.

3. Acetaldehyde and Acrolein (Minimum of 3 cigarettes)

The chromatographic column retaining the acetaldehyde and acrolein will be warmed at a controlled rate by the chromatographic temperature programmer to elute the gas phase components. The column used for trapping and analysis will correspond to that of Laurene, et al (7,8).

4. Formaldehyde (Minimum of 6 cigarettes)

Formaldehyde will be determined spectrophotometrically by the method described by Newsome et al (9,12). See Appendix A.

5. Hydrogen Cyanide (Minimum of 3 cigarettes)

The cyanide content of the ascarite extract will be determined using the colorimetric procedure described in Tob. Sci. XIV, 12-15, January 16, 1970 (10). See Appendix A.

6. pH (Minimum of 3 cigarettes)

The pH of the gas phase of smoke will be determined using the smoking chamber and combination electrode described by Sensabaugh (15).

C. Smoke Condensate

1. Water and Acetone (Minimum of 0.3g condensate, 12 cigarettes)

The method of Schultz and Spears (17) has been optimized to allow the simultaneous determination of water and acetone in a dioxane suspension of the condensate. The determinations will be carried out using a Porapak Q column for the separation and absolute ethanol as the internal standard.

2. Nicotine (Minimum of 0.3 g condensate, 12 cigarettes)

Nicotine will be determined by the method of Mumpower and Kiefer (11). Conditions will be chosen such as to allow the simultaneous determination of nornicotine.

3. "Tar"

The "tar" content of the condensate will be determined by subtraction of determined weights of water and nicotine in the acetone-condensate suspension from the weight of condensate corrected for acetone content.

4. Phenol, o-cresol and m- + p-cresol (Minimum 0.3 g condensate, 13 cigarettes)

Phenol, o-cresol and m- + p-cresol will be determined by steam distilling the sample from an acid medium using a Griffith Still, extracting the phenols into ether and injecting aliquots of the extract onto a Casterwax gas chromatography column. The procedure will incorporate ^{14}C -phenol as a tracer and correspond to that of Oakley et al (13).

5. pH (Minimum of 0.3 g condensate, 12 cigarettes)

The condensate preparation will be contacted with sufficient CO_2 -free water to yield a sample containing 10 mg per ml of condensate. The pH of the sample will be measured using a combination electrode. The procedure is comparable to that described by Grob (5).

6. Weak and Very Weak Acids (Minimum of 0.3 g condensate, 12 cigarettes)

The weak and very weak acids of condensate will be determined by potentiometric titration with tetrabutyl ammonium hydroxide in pyridine. See Cundiff, Sensabaugh, and Markunas (3).

7. Phenanthrene, Benz(a)anthracene and Benzo(a)pyrene (Minimum of 6 g of condensate, 200 cigarettes)

Phenanthrene, benz(a)anthracene, and benzo(a)pyrene will be determined using a modification of the method of Wynder and Hoffman (20,pl65).

The method will utilize liquid scintillation counting of ^{14}C -phenanthrene, -benz(a)anthracene, and -benzo(a)pyrene as measures of the recovery of the hydrocarbons in the analysis. Final measurement of benz(a)anthracene and benzo(a)pyrene will be by fluorescence spectrometry. Phenanthrene will be measured gas chromatographically if conditions can be found to adequately resolve it from anthracene.

8. Dibenz(a,j)acridine (Minimum of 6 g condensate, 200 cigarettes)

The method of Van Duuren (19) will be applied to this analysis on a non-routine basis.

V. Network of Operations and Work Schedule

A realistic network of operations and work schedule must reflect the manner in which samples are available. The schedule should also produce sufficient data for each cigarette sample to allow a tentative chemical "ranking" of the samples in a minimum period of time. Cigarettes should be analyzed early in the analytical program to minimize the influence of sample storage on the composition of the smoke. Sample storage is less a problem for condensates because new condensate will be generated regularly. With these factors in mind we plan first to determine the "tar" and nicotine deliveries of each cigarette sample, second to complete the gas phase analyses for each cigarette and finally to carry out the chemical analyses of submitted and freshly generated smoke condensates. The time-limiting factor in the determination of "tar"/nicotine and gas phase components is the smoke generation hardware. In both cases, no more than four cigarettes can be smoked simultaneously. The time-limiting factor in the analyses of condensates is the determination of polycyclic aromatic hydrocarbons.

Tar and nicotine delivery

Analyses of the particulate matter generated by the experimental cigarettes under both high and low fume hood velocities have been completed. Results were submitted to the project officer on 6-29-70 and 8-24-70.

Gas phase delivery

Analyses of the acetaldehyde and acrolein deliveries of the experimental cigarettes have been completed. Results were submitted on 6-29-70 and resubmitted on 8-24-70. Analyses of the formaldehyde and hydrogen cyanide deliveries of the cigarettes are underway. Analyses of the carbon monoxide, carbon dioxide and oxides of nitrogen deliveries of the cigarettes are being initiated. It is anticipated that the results of these

determinations and of measurements of the pH of the gas phases will be available on approximately 11-1-70.

Condensates

Submitted and freshly generated condensates will be subjected to analysis immediately upon completion of the analyses of the cigarettes. It is anticipated that the analysis of four to eight samples of condensate will be completed each month. Some time will be devoted to satisfying specific requests of the Tobacco Working Group (such as the determination of dibenz(a,j)acridine on selected condensates), checking questionable results of cigarette analyses, and improving general methodologies. Efforts related to any of those tasks outlined in Appendix B which are of interest to the Tobacco Working Group would also be initiated in this time period.

VI. Reporting Results

Results will be reported using forms such as those presented in Appendix C. Form A will be completed for each analysis and kept on file at ORNL. A second format constitutes a summary of all analytical results for each sample code and will be updated and forwarded to the project officer periodically. A third form summarizes the results for each individual component in all of the samples and will be updated and forwarded to the project officer periodically.

Supplemental forms will be used to present results in units other than those included in the appended forms. We anticipate expressing results in the following units:

- a. "Tar" and nicotine - mg per cigarette, mg per gram tobacco burned, weight percent of TPM (water, nicotine)
- b. Gas phase components - microgram per cigarette, microgram per gram tobacco burned, microgram per liter of smoke.
- c. Condensate components - mg per gram condensate, microgram per cigarette, weight per cent (acetone water, dry condensate), milliequivalent per cigarette (weak acids, very weak acids, total acids).

Sufficient information will be on file to allow expressing the results in other desired units.

Results will be reported as the mean of at least triplicate determinations. The value of the standard deviation (σ) will be reported with the

mean. Sets of results for which the relative standard deviation is 5% or less of the mean will generally be accepted. Somewhat poorer agreement will be accepted in cases where systematic errors are unavoidable, e.g. poorly packed cigarettes, and single-cigarette analyses. Better agreement, relative standard deviations of less than 5%, will be sought in other cases, e.g. nicotine alkaloids in TPM. Early results will continuously be reviewed in the light of later results and analyses will be repeated if indicated worthwhile by the review.

Results of tar-nicotine analyses were forwarded to the project officer on 6-29-70 and 8-24-70. Results will be updated and forwarded to the project officer periodically.

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APPENDIX A — ALTERNATE PROCEDURES

The following procedures are presently under consideration as alternate methods for the stated analyses. Major modifications and new procedures will be applied only after approval by the Tobacco Working Group.

1. Oxides of Nitrogen

The colorimetric Saltzman procedure described by Newsome et al (12) has been studied extensively as a method for the quantitative determination of oxides of nitrogen. We find the colorimetric procedure excellent but are not satisfied with trapping efficiencies. We have considered single and series arrangements of our standard trap system, Nesbitt bubblers, Milligan bubblers, and frit-containing gas dispersion traps and regularly detect approximately 25% of the "theoretical" NO/NO₂ content (as judged from published data of the NO-NO₂ content of typical cigarettes) of the cigarette.

Our experiences indicate that the individual-puff system described by Newsome et al (12) would give acceptable results. The labor involved in applying the individual-puff technique to the 23 sample cigarettes as well as the anticipated difficulty of adequately reproducing the technique for each cigarette have necessitated our consideration of alternative procedures.

The method of Barkemeyer and Seehofer (2) has been considered and temporarily rejected because of the large sample required (minimum of 45 cigarettes for a triplicate analysis). The state-of-art method described by Urbanic (18) for the direct determination of NO in the gas phase of cigarette smokes is of great interest but has been temporarily rejected as requiring an excessive expenditure of time and money. Procedures developed in-house have been considered but are not sufficiently proven to justify their application to these initial analyses.

The Saltzman procedure can be conveniently applied to the analysis of the entire delivery of cigarettes provided an apparatus can be constructed which allows the collection, without loss, of the entire delivery in such a way as to provide time for the air oxidation of NO to NO₂. Such an apparatus has been described by Murie (10). Puffs are taken by syringe and the content of the syringe is expelled into an evacuated collection flask.

We intend to employ the method described in the body of this protocol unless rejected by the Tobacco Working Group. Should the results prove inadequate ample time exists for a repeat of the determinations of the oxides of nitrogen using any other procedure.

2. Acetaldehyde and Acrolein

In the method described by Laurene et al (7,8), the gas phase is condensed in a cold trap and the contents of the trap are transferred to a gas chromatography column. We are able to eliminate the transfer step by condensing the gas phase directly on the head of the gas chromatography column. In operation, the gas phase is drawn into a gas-sampling loop during the puff and the contents of the loop are swept by helium onto the column held at -75°C between the puffs. When smoking is completed, the column temperature programmer warms the column at a selected rate until acetaldehyde and acrolein emerge and are recorded.

3. Formaldehyde

In the procedure summarized by Newsome et al (12), trapping of formaldehyde was carried out in a 0.1 per cent solution of chromotropic acid. It was found that the trapping efficiency of the chromotropic acid was inadequate when contained in traps of the configuration we have adopted. In agreement with the information supplied us by Iyerly (9), we found that the incorporation of a sodium metabisulfite solution as trapping agent yielded more precise results. The overall determination remains essentially as described by Newsome et al (12).

4. Hydrogen cyanide

The electrometric method of Baker and Morrison (1) described by Newsome et al (12) has been investigated at length and rejected as unreliable in our hands. We have found the technique too greatly influenced by experimental conditions and have experienced inordinate difficulty in generating a reliable calibration curve.

Methods generated in-house for the determination of HCN in the gas phase of smokes appear very promising. In one case, the cyanide content of the trap is determined polarographically while in another case, a cyanide ion-specific electrode is employed for the determination. The ion-specific electrode procedure has consistently produced higher than anticipated results but shows particular promise. Neither method is sufficiently proven to justify its application in these initial analyses.

The method summarized in the body of this protocol has been examined and found to produce values for HCN in good agreement with published results and of good precision. We intend to employ this method unless rejected by the Tobacco Working Group. Should the results prove inadequate

for the purposes of this program, ample time exists for a repeat of the determinations of HCN using any procedure.

An alternative method for the determination of the hydrogen cyanide delivery of cigarettes has been supplied to this group by Lyster (9). In this case, smoke is drawn through traps containing sodium biphosphate, hydroxylamine hydrochloride and acetic acid. The cyanide content of the traps is determined by the method of Schilt (16). This method has been found to provide results in good agreement with those from the ascarite method but is considerably more laborious.

APPENDIX B — POSSIBLE ADDED RESPONSIBILITIES

The following problems will receive only as much attention as can be allotted without interfering with the generation of the requested data. None of these problems will be elevated to a priority status comparable to that of the requested analyses unless specifically requested by the Tobacco Working Group.

1. Correlation with biological testing of the gas phase.

A study of the operating protocol of A. D. Little, Inc., and discussions with Dr. P. Thayer who is involved in that work have revealed two areas of probable poor correlation between the analytical work carried out at ORNL and the gas phase tests carried out at A. D. Little, Inc. In one case air is forced through the cigarette rather than drawn through the cigarette. In the other case, an aqueous solution of condensed smoke is tested. Neither condition has a strictly comparable analytical counterpart. Should a closer correlation be deemed advantageous, sample generation and treatment methodologies mimicing those employed at A. D. Little, Inc. could be established at ORNL.

2. Improved gas phase analyses.

Methods available for the quantitative determination of formaldehyde and oxides of nitrogen in the gas phase of smoke yield values related to the compounds of interest but not specific for these compounds. New gas chromatographic packings and our interfaced smoking machine — cryothermal gas chromatograph offer a possible solution to this problem. If more accurate results are needed for these components, the utility of the gas chromatographic approach can be examined using existing equipment.

3. Carboxylic acids in smoke condensate.

Our operating protocol defines three separate test procedures presumably related to the acidity of condensates and/or the gas phase of smokes. It would appear that a quantitative determination of even the simplest acids would be of value. Our experience suggests that a gross determination of the major carboxylic acids in smoke condensate can be made gas chromatographically. Such a procedure is to be described (10) by Tennessee Eastman researchers at the forthcoming Tobacco Chemists Conference. The procedure can be made routine for the cost of a simple gas chromatograph.

4. N-nitrosamines

Speculation about the involvement of N-nitrosamines in tobacco-related health hazards suggests that N-nitrosamines be included in the analytical work. A brief consideration of the utility of the nitrogen-specific detection of gas chromatography effluents and of polarographic methodologies would allow an assessment of the possibility of routinely assaying smoke condensate and/or whole smoke for N-nitrosamines.

5. Ageing of smoke

Critics of some biological test procedures indicate that smoke which has been in contact with a biological system for more than several seconds is no longer comparable to smoke which is inhaled and quickly exhaled. Our cryothermal gas chromatography system and element specific detectors may provide a means to establish the significance of short ageing times on the chemical characteristics of smokes.

6. Sample storage

The rather long duration of this contract affords an opportunity to establish the influence of storage time and storage conditions on the chemical characteristics of smokes and smoke condensates. No additional equipment would be required for this study. Additional operator time for repeating selected analyses at appropriate times is all that would be necessary.

APPENDIX C — REPORT FORMAT AND FORMS

Results will be submitted using forms of the type appended here. Each submission will include results reported earlier plus the results of analyses completed since the previous submission. Each submission will thus be complete and supercede all previous submissions.

Tables presenting the ranking of the samples according to the content of each component will be included with the submissions. Results will be expressed in other desired units and/or calculated in other ways upon request.

Cover pages will be included which define the methods of calculation and describe any special features of the samples and/or analysis.

Comparable forms will be submitted which summarize the results of other analyses of the 23 experimental cigarettes.

Experimental Characteristics

Sample Code:

Reporting Date: _____

Analyses:

I. Sample Description and History

1. Description
2. Date manufactured
3. Date received
4. Date sealed and frozen
5. Time thawed
6. Date opened to conditioning
7. Time conditioned
8. Laboratory temperature during conditioning
9. Laboratory humidity during conditioning
10. Date aliquots taken
- 11.
- 12.
- 13.
- 14.
15. Comments

II. Smoke Generation and Collection

1. Date smoked
2. Smoking machine
3. Laboratory temperature during smoking
4. Laboratory humidity during smoking
5. Puff volume
6. Puff duration
7. Puff frequency
8. Butt length
9. Total number of cigarettes smoked
10. Average weight of cigarettes
11. Average number of puffs per cigarette
12. Number of cigarettes per pad
- 13.
- 14.
15. Comments

III. Analyses

1. Date of determination
 - a.
 - b.
 - c.
 - d.
2. Method used for determination
 - a.
 - b.
 - c.
 - d.
3. Comments

COMPLETED ANALYSES

Reporting Date:

Last Report:

Date Analyzed:

Sample:

I. Particulate Matter

	<u>mg/cig.</u>	<u>S</u>	<u>mg/g.tob.</u>	<u>S</u>	<u>Wt% TPM</u>	<u>S</u>
1. TPM	_____	_____	_____	_____	_____	_____
2. Water	_____	_____	_____	_____	_____	_____
3. Nicotine	_____	_____	_____	_____	_____	_____
4. "Tar"	_____	_____	_____	_____	_____	_____

II. Gas Phase

	<u>µg/cig.</u>	<u>S</u>	<u>µg/g tob.</u>	<u>S</u>	<u>µg/l.smoke</u>	<u>S</u>
1. CO	_____	_____	_____	_____	_____	_____
2. CO ₂	_____	_____	_____	_____	_____	_____
3. NO ₂	_____	_____	_____	_____	_____	_____
4. Acetaldehyde	_____	_____	_____	_____	_____	_____
5. Acrolein	_____	_____	_____	_____	_____	_____
6. Formaldehyde	_____	_____	_____	_____	_____	_____
7. HCN	_____	_____	_____	_____	_____	_____
8. pH (average, as defined	_____	_____	_____	_____	_____	_____

Puff	1	_____	_____
	2	_____	_____
	3	_____	_____
	4	_____	_____
	5	_____	_____
	6	_____	_____
	7	_____	_____
	8	_____	_____
	9	_____	_____
	10	_____	_____
	11	_____	_____

III. Smoke Condensate, Melpar, Generated on _____

Units:

1. Acetone	_____
2. Water	_____
3. Dry Condensate	_____

Units:

3. Wet Condensate	_____
4. Dry Condensate	_____

Units:

5. Nicotine	_____	_____
6. Nornicotine	_____	_____
7. Alkaloid as Nicotine	_____	_____
8. Phenol	_____	_____
9. O-Cresol	_____	_____
10. M-Cresol + P-Cresol	_____	_____
11. Phenanthrene	_____	_____
12. Benz(a)anthracene	_____	_____
13. Benz(a)pyrene	_____	_____
14. Dibenz(a,j)acridine	_____	_____
15. Weak Acids (as acetic)	_____	_____
16. Very Weak Acids (as phenol)	_____	_____
17. "Tar"	_____	_____
18.	_____	_____
19.	_____	_____
20.	_____	_____

Units:

21. Weak Acids	_____	_____
22. Very Weak Acids	_____	_____
23. Total Acids	_____	_____

Units:

24. Water	_____
25. Alkaloids, as Nicotine	_____
26. "Tar"	_____
27. pH	_____

IV. Smoke Condensate, ORNL, Generated on _____

Units:

1. Acetone	_____	_____
2. Water	_____	_____
3. Dry Condensate	_____	_____

Units:

3. Wet Condensate	_____	_____
4. Dry Condensate	_____	_____

Units:

5. Nicotine	_____	_____
6. Nornicotine	_____	_____
7. Alkaloid as Nicotine	_____	_____
8. Phenol	_____	_____
9. O-Cresol	_____	_____
10. M-Cresol + P-Cresol	_____	_____
11. Phenanthrene	_____	_____
12. Benz(a)anthracene	_____	_____
13. Benz(a)pyrene	_____	_____
14. Dibenz(a,j)acridine	_____	_____
15. Weak Acids (as acetic)	_____	_____
16. Very Weak Acids (as phenol)	_____	_____
17. "Tar"	_____	_____
18.	_____	_____
19.	_____	_____
20.	_____	_____

Units:

21. Weak Acids	_____	_____
22. Very Weak Acids	_____	_____
23. Total Acids	_____	_____

Units:

24. Water	_____
25. Alkaloids, as Nicotine	_____
26. "Tar"	_____
27. pH	_____

SUMMARIZED RESULTS:

Reporting Date:

Last Report:

Date Analyzed:

Samples:

Units:

SAMPLE	TPM		WATER		NICOTINE		TAR	
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								

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