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TOBACCO SMOKE RESEARCH PROGRAM PROGRESS REPORT

M. R. Guerin
P. Nettesheim

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and
BIOLOGY DIVISION

TOBACCO SMOKE RESEARCH PROGRAM PROGRESS REPORT

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SUMMARY

The Tobacco Smoke Research Program is composed of two separate but related projects--the tobacco smoke chemistry project and inhalation bioassay methodology project. The objective of the chemistry project is to provide both chemical and hardware support to NCI projects directed at the elucidation of characteristics of less hazardous smoking products. Specific efforts include the generation of chemical data to characterize smokes undergoing biological testing elsewhere, development and evaluation of methodologies specified by NCI protocols, research into means for the more complete characterization of smokes, and short-term support of special NCI projects. The goal of the inhalation project is to develop new methods of assessing the relative carcinogenicity of tobacco smoke. Specific efforts include an evaluation of nose-bypass techniques, considerations of novel smoke exposure protocols, selection of preferred target animal species and/or strains, development of means for earlier endpoint detection, design and fabrication of exposure hardware, determination of respiratory characteristics under exposure conditions and studies of smoke dosimetry.

Tobacco Smoke Chemistry. Final data have been generated on the first series of primary experimental cigarettes for the twenty-five components specified by the NCI. Data for preliminary study are also available for whole smoke hydrogen cyanide, palmitic acid, oleic, linoleic, plus linolenic acids, stearic acid, isoprene, nickel, cadmium, lead, chromium, zinc, and mercury. Methods for computer data treatment and formating have been developed and used to prepare the data submission. Data generation is nearing completion on a series of experimental filter cigarettes and a high nicotine-low nicotine experimental pair. Data have been reported for a series of cigarettes containing ammonium sulfamate and for marijuana cigarettes. The second series of primary experimental cigarettes is now being examined.

Interlaboratory comparisons of data and NCI-specified procedures for fifteen components revealed that procedures for the determination of hydrogen cyanide, oxides of nitrogen, and polycyclic aromatic hydrocarbons

were in need of further study. A collaborative study of condensate sampling methodologies demonstrated the degree to which sampling affects lot-to-lot chemical reproducibility and suggests improvements in protocols for subsequent work. Methods development and evaluation efforts have provided absolute results for acetaldehyde and acrolein, a carbon monoxide-carbon dioxide procedure requiring one-half the time of the previous procedure, substantially greater recoveries in the PAH procedure, a viable hydrogen cyanide procedure applicable to both gas phase and whole smoke, a superior gas chromatographic column for routine phenol determinations, and alternative sampling and dry condensate procedures.

Research activities emphasize the development of methodologies for the more complete characterization of smokes. Chromatographic component profiling continues to afford the best approach to greatly expanding the number of components routinely surveyed for correlation with bioassay results. The profiling technique has provided a convenient method for estimating isoprene deliveries of cigarettes. A procedure for determining 14-18 carbon free fatty acids has evolved from studies of TPM profiling. Sulfur components can be profiled directly on as little as 100 μl of gas phase. A method for the quantitative determination of major sulfur gases has been developed and its application demonstrates that popularly held views as to sulfur delivery are grossly in error. Spark source mass spectrometry, particularly using isotope-dilution, has been found very well suited to determinations of trace metals, including nickel, in condensates. Recent evaluation of the Coulson conductivity detector has demonstrated its utility for easily surveying major amines and nitriles in smokes. Improved, convenient methods for determining NO free of NO_2, HCN, and nitrogen-heterocyclics are likely outgrowths of nitrogen profiling.

Other activities include the fabrication of smoking devices for routine assays. One device expands the number of TPM or gas phase samples that can be generated. The other, designed to allow sample averaging for analyses of single composite puffs of very fresh smokes affords an alternative route to generating acetaldehyde, acrolein, isoprene, carbon oxides and nitrogen oxides samples. A special study of dog inhalation methodologies has included the fabrication of six exposure machines for pilot studies and a chemical evaluation of the machine relative to "dog puffing" and standard smoking.

Inhalation Bioassay Methodology. Nose bypass devices were developed to increase smoke deposition in exposed hamsters to tobacco smoke. The device most thoroughly tested to date is a laryngeal cannula. Studies with radioactive smoke showed a two-three fold increase in smoke particle deposition over that seen in nose breathing hamsters. Respiratory physiology studies showed that this effect was in large part due to altered tidal volume and respiratory frequency. A permanent tracheotomy is being developed as an alternative to the laryngeal cannula.

Chronic smoke exposure schemes for nose breathing hamsters were designed and tested with the intermittent ORNL smoking machine. As many as twelve cigarettes per day are tolerated by the animals if enough time is allowed between cigarettes for detoxification. Animals exposed for six-eight months using one of these smoke exposure schemes showed significant changes of the laryngeal and bronchial epithelium. Similar tests are now being conducted with the Hamburg II smoke generator.

Six different hamster strains were tested for their relative susceptibility to tobacco smoke toxicity; one of the strains showed considerably greater resistance than the other five and will be tested further for susceptibility to the carcinogenic effects of smoke. Studies are currently underway to develop smoke exposure schemes for rats which were shown to be highly susceptible to the carcinogenic effects of polycyclic hydrocarbons and smoke condensate.

TOBACCO SMOKE CHEMISTRY PROJECT

1.00 INTRODUCTION

The primary function of this project continues to be the generation of data for the chemical characterization of the smokes of experimental cigarettes. Quantitative determination of selected components of particulate matter, gas phase, and condensate provides the core chemical data. Special components are determined in selected cigarette smokes as needed.

This report period has also included a thorough examination of analytical procedures. Nicotine, water, TPM, tar, TPM phenolics, whole smoke and gas phase hydrogen cyanide, gas phase carbon dioxide, carbon monoxide, oxides of nitrogen, acetaldehyde and acrolein have been considered in several interlaboratory collaborative studies. Polynuclear aromatic hydrocarbons have been considered in an informal collaborative study. The large number of completed analyses have allowed the computation of limits of precision for each procedure. Observations important to improving initially specified protocols have been recorded. Some improvements have been implemented while others are under consideration.

Exploratory studies of novel analytical techniques for the further chemical characterization of smokes are carried out as possible. Studies of the sulfur components of smokes continue to demonstrate the questionable nature of generally held views as to the quantity and distribution of these components. Component profiling of particulate matter and condensate has produced a convenient procedure for the rapid estimation of free fatty acids. Studies of the acetaldehyde-acrolein procedure have produced a convenient means of determining isoprene deliveries. A basic methodology has been established for measuring trace metals, including Ni and Cd in condensates. Recent work with the nitrogen specific gas chromatography detector suggested the possibility of significant improvements in methods for measuring NO free of NO_2, HCN, and nitrogen heterocyclics.

The final area of expanded effort is that involving "special studies". Special-interest cigarettes--a pilot production of filter cigarettes, low and high nicotine cigarettes, ammonium sulfamate treated cigarettes, and

marijuana cigarettes--have been the subject of regular analyses and special analyses. The chemical and hardware capabilities of this project have been applied to a study and modification of dog inhalation methodologies.

This report summarizes the activities in progress from approximately July 1, 1971 to January 1, 1973.

2.00 SAMPLING AND SAMPLE PREPARATION

Sample generation and sample preparation techniques are singularly critical to the reliability and significance of every chemical assay constituting this control facility. An error of a single puff during analytical smoking significantly alters the per cigarette delivery of the measured component. Poorly reproducible or poorly representative aliquoting of submitted smoke condensate suspensions drastically reduces the utility of the condensate assay. These problems, characteristic of all smoke chemistry studies, are complicated by the fact that we deal with experimental and therefore less than typically uniform cigarettes. The fact that many of the first series cigarettes are very similar in delivery of most components requires added care to allow discriminating one product from another.

Sampling techniques specified in initial protocols have been under continuous study to assess their reliability and identify possible sources of error. Steps have been taken to improve the procedures where shortcomings have been identified. Steps have also been taken to extend sample generation capabilities.

2.10 Cigarette Smokes

Freshly generated smokes are required for the determination of total particulate matter, water, nicotine alkaloids, tar, carbon monoxide, carbon dioxide, acetaldehyde, acrolein, hydrogen cyanide, formaldehyde, oxides of nitrogen, and gas phase acidity. Several research and development projects also require that fresh smokes be generated. Steps have been taken to increase our sample generation capacity and to improve standard smoking conditions.

2.11 Smoking Machines

Smoking machines in routine use were described previously [13,15]. They include a four-port version of the standard Philip Morris twenty-port

smoker, a Filamatic pump system modified for the collection of the full gas phase delivery of a cigarette, and a vacuum pump system for routing the full gas phase delivery of a cigarette onto the head of a cooled chromatographic column. The four-port machine is used for the tar-nicotine determinations and, after appropriate modification, for the preparation of samples for formaldehyde and hydrogen cyanide determinations. Research efforts requiring the availability of standard particulate matter also depend on the four-port machine.

An additional smoking machine was needed to lessen the scheduling difficulties associated with the heavy usage of the four-port machine. Our requirement was for a multiple-port smoking device capable of generating standard particulate matter for special studies and amenable to the collection of gas phase samples for any analyses requiring the use of a trap down-stream of the Cambridge filter.

A six-port machine (Fig. 2-1) was designed and constructed which meets our requirements. The machine features a single Filamatic pump to smoke the six cigarettes in sequence. At time zero, all but one solenoid are closed. A standard two second puff is taken on the cigarette in line with the open solenoid. Ten seconds after the puff of the first cigarette is initiated, the pump is again activated and cigarette number two is puffed. The sequence repeats with each cigarette being smoked at a rate of one puff per minute, at intervals of ten seconds apart.

The use of a Filamatic pump, the same puffing mechanism that is used on the standard four-port machine, provides the typical puff profile. The number of puffs required to reach the 23 mm butt has been the same with the six-port machine as it has with the standard machine (Table 2-1). We anticipate no difficulty with adapting the machine to the collection of selected gas phase samples. Superior control of butt length and draft necessitates the continued use of the standard machine for tar-nicotine determinations. The six-port machine is presently in use for generating special samples--free fatty acids, TPM phenolics, inorganics, and whole smoke hydrogen cyanide.

A modified version of a "hexapuffer" [17,24] for sample averaging was also designed and fabricated. The machine (Figs. 2-2 and 2-3)

consists of a six-way gas sampling valve with a 5 ml loop placed exactly in the middle of a 205 ml copper tubing trap. The input to the trap consists of a hexagonal turret with six ports, each of which is equipped with an orifice to control the puff volume to 35 ± 0.5 ml. The six ports are connected to the trap via a common manifold and a Skinner valve. A 60 second timer with cam-actuated micro switches controls the duration of the puff and re-evacuation of the trap via a third port of the Skinner valve.

Cigarettes are lighted in sequence, one each minute. A stepping switch recycles the puffing-evacuation sequence through six puffs and on the seventh puff activates the six-way gas sampling valve, routing the contents of the loop to a gas chromatograph. If a cigarette requires more than eight puffs to burn to a 23 mm butt length, a switch that bypasses the stepping switch allows any number of puffs to be taken prior to activation of the stepping switch. This feature insures that a sample can be taken from the middle portion of any cigarette.

This device allows the analysis of very fresh smokes and provides a discrete sample compositionally representative of the full delivery of a cigarette. It is presently in use for studies of sulfur compounds and under consideration for carbon monoxide and nitrogen oxide analyses.

2.12 Smoke Generation Methodology

Proper smoke generation requires that smoking be carried out under standard conditions of environmental temperature and humidity and that cigarettes be properly conditioned and properly selected from each batch. An Aminco-Aire system has been in operation for approximately nine months to provide superior temperature and humidity control in the smoking-conditioning room. Although the unit is operating near its capacity and occasionally requires adjustment, environmental control has been much more stable than prior to its installation.

Cigarettes are now being selected according to resistance-to-draw (RTD) as well as by weight. A new pressure drop tester (Filtrona, Cigarette Components Limited) makes RTD selection practical. A larger population of cigarettes can now be practically surveyed for weight selection with the availability of a printing automatic digital balance.

The overall operating reliability of the smoking machines is now being routinely assessed by smoking highly characterized cigarettes each day analyses are performed. The Kentucky Reference Cigarette (1R1) and/or an inhouse secondary monitor cigarette (RJR) serves to test the machine. A difference of greater than 0.5 in the number of puffs required to reach the 23 mm butt mark is taken as evidence that additional adjustment of puff characteristics or hood velocity is necessary.

Cigarette storage and handling facilities have been improved. The freezer used for cigarette storage, normally maintained at -2°F, is now equipped with a continuously monitored alarm which activates when the temperature reaches +8°F. Steps can be taken immediately to stop the temperature rise and repair the malfunction. Cigarettes are now conditioned at 75°F and 60% r.h. for at least 48 hours before weight and RTD selecting.

The single departure from standard smoking characteristics during sample generation for tar-nicotine determinations is the use of the four-port machine as opposed to a twenty-port machine. Collaborative and special studies (see sections 3.10, 3.20) demonstrate that this is not a serious problem.

2.20 Smoke Condensate Suspensions

Cigarette smoke condensates analyzed here are received as acetone suspensions from a collaborating contractor [27] whose prime responsibility is to prepare condensates for mouse skin bioassay studies. The samples received are theoretically equivalent to that forwarded to the bioassay contractor. Consideration of the large quantity of data now generated on condensate samples suggested that sample preparation and distribution procedures in use here and by the production group [27] were in need of added study.

The initial approved protocol for the production group involved the combination of the contents of several large volume traps into a single flask by the rotary evaporation of the acetone used for transferring the collected condensate. The combined condensate was analyzed for water and acetone and adjusted to approximately 50 wt % condensate, 5 wt % water, 45 wt % acetone by the addition of water and/or acetone as needed. The 50 wt % suspension was then transferred with slurrying into a series of

small mouth two-ounce shipping bottles. One bottle was submitted to ORNL while the rest were forwarded for biological testing.

When aliquots were needed for analysis here, the bottles were thawed while gently shaken on a mechanical shaker. A Teflon stirring bar was placed in the suspension and the suspension was vigorously stirred while aliquots were quickly removed with a large bore transfer pipette. The aliquots were immediately exhausted into tared bottles and capped. The sample weight was determined gravimetrically and dry condensate composition by gas chromatographic analysis for water and acetone. Sufficient aliquots were withdrawn to provide samples for each determination that constitute this facility's responsibility.

There are obvious dangers with both protocols. In the case of the production protocol, it is difficult to impossible to insure that the condensate, present as a very viscous largely inhomogeneous slurry, is evenly distributed from bottle to bottle. Excellent care has been exercised to minimize inhomogeneous distribution and we expect that the majority of the samples submitted are representative of the whole. However, it is highly likely that inhomogeneity has occurred occasionally and that the sample submitted for chemical study did not adequately represent the overall production batch. This problem is serious because only two lots from the production series were scheduled for chemical study.

The same danger, poorly representative sampling, is associated with the aliquoting procedure. Measures of total alkaloids and colorimetric phenols, initially specified as estimates of sampling integrity, agree quite well over the four aliquots taken for each analysis. Values for nicotine content were found relatively unaffected by sample size. Both observations suggest that sampling has been reproducible. However, the fact remains that the aliquots must be drawn quickly from a questionably uniform slurry and the possibility of an occasional nonrepresentative sampling is very real.

A problem of particular concern is the relevance of the dry condensate estimate and the sample homogeneity test to the samples drawn for polynuclear aromatic hydrocarbon (PAH) analysis. Most parameters measured require sample sizes of between 100 and 300 mg. The samples for

PAH determinations generally run to approximately 6 g. The continued transfer of condensate using the small pipettes affords considerable opportunity for acetone to evaporate and consumes the great bulk of the submitted sample. Table 2-2 summarizes the results of a study of the aliquot to aliquot variability of the PAH aliquots and of the relevance of the dry condensate composition determined by analysis of a small aliquot. It is seen that the results can be quite good, as theoretically they should, but that isolated cases do occur when aliquot to aliquot variability is unacceptable and the theoretical dry condensate composition is in error.

Action has been taken to improve the sample handling procedures. Samples for chemical analysis are now withdrawn by the production group at a point after the trap contents are combined but before complete evaporation of the acetone and distribution into shipping bottles. The condensate sample is withdrawn as a substantially more dilute and, thus, better dispersed suspension from the entire production batch. The suspension is dried by rotary evaporation, frozen, and submitted for analysis.

The aliquoting procedure has also been changed. The dried condensate is thawed and sufficient acetone is added to produce a suspension approximately 25 wt % in dry condensate. The suspension is quantitatively transferred into a large volume separatory funnel equipped with a side arm for introducing the sample, a mechanical stirrer, and a Teflon stopcock. The stirrer vigorously mixes the suspension while aliquots are withdrawn via the stopcock. The results of a study of the dry condensate composition determined gravimetrically following acetone evaporation, as a function of sample size and point of withdrawal are summarized in Table 2-3. The data are taken as preliminary evidence that the new procedure is providing aliquots representative of the whole.

As an added refinement, samples for analysis are taken alternately rather than in series. An aliquot is withdrawn for water-acetone determination, then phenol determination, PAH determination, nicotine determination and so forth until the second water-acetone aliquot is obtained and the sequence is repeated. Each of the four aliquots taken for each analysis is, thus, obtained with a substantially different depth of suspension present.

An experiment was initiated with the cooperation of Dr. Patel of Meloy Laboratories to experimentally evaluate the variability possible from the sample distribution process alone. A comparison with a new sampling protocol was included in the experiment. Condensate was generated and removed from the trap as normal. When the dry condensate composition approached 25 wt %, an aliquot was withdrawn to provide a composite sample, and the acetone was removed completely. The remainder of the suspension was treated as earlier, eventually producing six bottles containing approximately 50% dry condensate suspension. The composite and individual bottles were forwarded for chemical analysis.

The results of this study are summarized in Table 2-4. Columns 1-6 present the results for each parameter found in each individual bottle. The average of the six values and the results found for the composite sample are also given. The column headed "Δ1-6" is the difference between the two poorest agreeing values expressed as a percent of the averages of those values. Two values are given for o-cresol since the o-cresol value for bottle 4 is unusually low. The ratio of the concentrations found for the acetone suspensions versus the composite is also given.

The variability is expressed in terms of the difference as a percent of the average only because this means was used to express the lot-to-lot variability in runs I and II where only two values were available. Agreement across the six bottles is reasonably good for all parameters. However, comparing the two values in poorest agreement reveals differences approaching those found in comparing runs I and II of some of the original samples. These differences are substantially greater, by approximately a factor of two, than differences found by analyzing a given bottle twice. This is taken as evidence that the sample distribution procedure represents a major contribution to the lot-to-lot variability experienced in the chemical results generated to date. It must be emphasized that this experiment considers variations only in sample handling and analytical techniques on a given batch of condensate. Differences in sample generation (smoking) and sample handling from production to production would be expected to increase the variability significantly.

The primary conclusion of this study is that lot-to-lot variability will continue to be large without modifications in sample handling and/or

scheduling protocols. Although an occasional erratic chemical result is unavoidable, the precision with which the analyses can be executed on a given sample makes relatively little contribution to the variability normally experienced in condensate studies.

The final important observation from the special sampling study is that the composition of the composite, dried sample is generally different from that of the acetone suspension by a factor of approximately 1.1 (last column). This is important because the results of the repeated analysis of series one condensates, using composite rather than distributed samples, have also been found to differ by approximately the same ratio. We must expect the results for at least some components to differ from those found in the acetone suspensions if we change the protocol to analyses of composite samples.

The complete first series of cigarettes have been smoked again to provide condensate samples strictly for chemical testing. The samples have allowed the generation of a third value for each parameter. This simple addition to the initial protocol may be the single most important as it provides a basis for identifying the suspect result for those cases where the two already generated values are widely differing. The new samples have also allowed the application of the greatly improved PAH assay procedure to the first series of cigarette smoke condensates.

3.00 ROUTINE CHEMICAL ASSAYS

The routine chemical analysis of the smokes delivered by the National Cancer Institute experimental cigarettes constitutes the core of the Tobacco Smoke Analysis Project. The data generated here are to be correlated with both the characteristics of the cigarettes and the results of bioassays. It is particularly important that each analytical procedure be defined as to the precision of the results obtained and the reliability of those results.

A greater variety of chemicals in smokes are being semi-routinely determined here than most laboratories. Restrictions of early protocols, notably the requirement that the full delivery of each cigarette must be trapped for gas phase analyses, and practical limitations in the number of cigarettes which can be analyzed further complicate the task. The application of a large variety of assay procedures often involving methods developed in-house makes it all the more important that every opportunity be taken to characterize each procedure. Each designated component of particulate matter, gas phase and smoke condensate has been determined in quadruplicate at least twice for each of the 23 cigarette types constituting the first experimental series. Analyses of condensates from the second experimental series of cigarettes has been initiated. A complete re-analyses of condensates from the first series of condensates has been completed. The smokes delivered by a pilot batch of filter cigarettes and by the second series of experimental cigarettes are under study.

The results of all analyses of smokes from series I cigarettes are to be studied in detail prior to presenting the final data report. Each analysis is being studied from a procedural stand point--percent recoveries and agreement between different methods of measuring essentially the same parameter--and a statistical stand point. Initial observations relating to analytical reliability are summarized in the following sections. Newly developed improvements in various procedures are also described.

3.10 Particulate Matter

Total particulate matter (TPM), water, nicotine alkaloids, and tar

are routinely determined by the classical approach of controlled smoking, gas chromatography, steam distillation and spectrophotometry, and calculating tar. The results are important for determining the gross delivery of the cigarette and useful for comparing the experimental product with commercially available cigarettes. Being the most carefully controlled and characterized procedure in tobacco smoke chemistry, its study is important in estimating the reliability of presently employed techniques. Agreement with collaborators' results means not only that the measurement techniques are properly executed but also that all prior steps--cigarette storage, handling, selection, and smoking--are properly carried out. This is particularly important because the same techniques are used for the generation of gas phase and special samples. If agreement is good for this analysis, poor agreement for other parameters can be traced immediately to limitation of the analytical measurement. The possible influence of the many variables associated with cigarette handling and smoking could be eliminated as a causative factor.

The best measure of the reliability of any analysis is obtained by comparing results generated by two or more laboratories. Tables 3-3, 3-4, and 3-5 summarize the results of such studies for several smoke components. Values are given for TPM, water, nicotine, and tar determined at ORNL and by three collaborating (industry) laboratories. Comparative study "number one" (Table 3-1, 3-2, 3-3) is truly collaborative in nature in that the cigarettes were prepared, selected, and conditioned by the collaborators specifically for comparative analytical testing. It differs from normal operations and the other comparative test in that the cigarettes were canned after conditioning and smoked immediately upon opening the cans without additional conditioning. Study "number two" (Table 3-4) is simply a comparison of the results generated here with those provided with the inhouse monitor. The cigarettes were handled as normal samples both here and by the collaborators. Study "number three" (Table 3-5) is a comparison of the results generated here with those generated by the collaborator on a series of NCI experimental cigarettes. The only thing in common for this study is that the samples came from the same production batch of (approximately 1,000,000) cigarettes.

Agreement between laboratories in results for TPM, nicotine, and tar has ranged from good to excellent. Agreement in results for water determinations is poorer but acceptable [6]. These data are taken as evidence that this assay, including the sample storage, handling, and selection procedures, is providing highly reliable results. The use of the four-port machine apparently does not produce results detectably different from those obtained by the standard twenty-port machine. The problem of a limited sample size, 16 to 20 smoked cigarettes, is apparently overcome by care in cigarette selection.

Pillsbury and Bright [28] have recently reported a bias in results for nicotine determinations when aliquots are taken from the pad extract rather than steam distilling the entire extract plus pad. An aliquot procedure is employed here. The comparative studies reveal no substantial difference between the results determined here and those determined by the collaborators. A longer extraction period, at least 16 hours here as opposed to 30 minutes used in the reported study, may account for the good agreement.

Tables 3-6, 3-7, 3-8, and 3-9 summarize the precision experienced with determinations of TPM, nicotine, water, and tar respectively. Each tabulated value is the average of the parameter listed for the 23 reported determinations of each component. The precision of both the first and second analysis are tabulated for results expressed in mg/cig., mg/puff, and mg/g of tobacco burned. The coefficient of variation, defined as the standard deviation (s) divided by the mean (m) expressed in percent ((s/m)100), provides a measure of port to port variability. The standard error of the mean, the standard deviation divided by the square root of the number of observations ($\sqrt{n}$) expressed as a percent of the mean

$((\frac{s/\sqrt{n}}{m})100)$ provides a measure of the variability of the mean. Although the value of two times the standard error is often used as an estimate of the 95% confidence interval of the mean, the true 95% confidence interval is considerably larger for the small number of observations involved. True confidence intervals expressed as a percent of the mean

$(100(t\ \frac{s}{\sqrt{n}}/m)$, where t is the appropriate "students t" factor for the

number of ports actually employed to produce the result, are also tabulated.

Note in comparing the values that the coefficient of variation is computed in terms of one standard deviation. The tabulated variation, then, would be expected to occur 60% of the time. A better measure of the port to port variability is derived by multiplying the coefficient of variation by two. Thus, port to port variability for 95% of the cases would be expected to range from 6-8% rather than 3-4%.

A study of these data shows that the precision for the second analysis is somewhat better than for the first analysis. Increased experience with this analysis and improved cigarette handling techniques may be manifest in this data. We also note that the precision of the results expressed on a per puff basis is somewhat poorer than those expressed as per cigarette or per gram of tobacco burned. This, too, may be expected since the per puff data require the use of another parameter--the number of puffs per cigarette, itself subject to error--for the computation.

The precision appears to be quite good for TPM, nicotine alkaloids and tar determinations. The precision of the water determinations is poorer but generally acceptable. A comparison of the precision of these determinations here with those for TPM, tar, and nicotine experienced elsewhere may be obtained by an examination of Table 3-10. Agreement is, again, quite good.

3.20 Gas Phase

The gas phase is defined for analytical purposes as that fraction of the whole smoke which passes through a standard glass-fiber (Cambridge) filter. Each gas phase component of interest in this program has been determined at least twice for each experimental cigarette. The acetaldehyde-acrolein system has been calibrated to provide reliable quantitative results. Shortcomings of the methods specified for determinations of hydrogen cyanide and oxides of nitrogen have been better defined. Summaries of the performance of the gas phase procedures follow.

3.21 Acetaldehyde and Acrolein

The problem of collecting the sample for the determination of acetaldehyde and acrolein in smoke has been a formidable one. Several methods have been proposed for sampling [20, 24] but only one of these fits the requirements of this program, namely, the collection of the gas phase of a whole cigarette. The method of Laurene et al [20] involves trapping the gas phase in a copper tube radiator trap immersed in liquid nitrogen. The contents of the trap are transferred to a gas chromatograph by immersing the trap in a hot oil bath, and sweeping the contents into the gas chromatograph with carrier gas. We have chosen to eliminate the need for a trap by collecting the gas phase at the head of a cryothermally cooled gas chromatographic column. After the cigarette has been smoked, the column is temperature programmed in stages set to elute the desired constituents as pure component peaks. This system eliminates most of the contact of the gas phase with possibly reactive hot metal surfaces.

Regardless of the system chosen for trapping the gas phase, losses of both acetaldehyde and acrolein occur. The percentage of loss varies with the type of containment material as well as the surface area of the material that comes in contact with the gas phase. We have sought to eliminate as much surface contact as possible by using unheated tubing of small diameter, and where possible, relatively inert materials such as glass.

The most critical problem in obtaining reliable results is that of preparing valid standards of acetaldehyde and acrolein. These compounds are very volatile as well as highly reactive. Acetaldehyde, with a B.P. of +20°C, is very difficult to measure with any standard pipet or syringe. Even when the acetaldehyde and the measuring device are chilled, accurate measurement is not possible if the acetaldehyde is pure. When acetaldehyde ages for an appreciable time, particularly at room temperature or above, polymerization takes place giving paraldehyde which boils at +124°C. If paraldehyde is present in significant amounts, the reagent becomes much easier to pipet because of decreased volatility, however, the acetaldehyde content is lower than expected and the calculated results for acetaldehyde in the gas phase will be high. We have tried weighing acetaldehyde in a septum-sealed vial, and diluting with a suitable solvent.

Such mixtures, especially in concentrations that would correspond to the acetaldehyde in the gas phase of a whole cigarette, were unstable. In any case, direct injection of acetaldehyde standards into the gas chromatograph always gave low results when the peak area of the standard was compared to the peak area of the gas phase sample. Several methods were tried for injecting acetaldehyde into the cigarette holder in order to sample the standard in the same manner as the cigarette, but all were unsuccessful due to the instability of the standard solutions.

Spears [34] suggested a method of obtaining reasonably accurate values for both acetaldehyde and acrolein standards. A five gallon carboy was equipped as shown in Figure 3-1. A few drops of acetaldehyde were added to the carboy and allowed to remain overnight to provide a homogeneous mixture of acetaldehyde, vapor and air. The mixture was calculated to contain approximately one mg of acetaldehyde when ten standard puffs were taken from the carboy by the smoking machine. Each puff was swept from the loop of the smoking machine onto the gas chromatogtaphic column which was cooled to -75°C in the same manner as for the gas phase of a cigarette. A second ten puffs were collected in water via tandem gas washing bottles with medium frits each containing 75 ml of H_2O. Colorimetric analysis [19] was performed upon aliquots of the water. Only 2.8% of the total acetaldehyde was found in the second bottle. The values obtained from the colorimetric analysis were applied to the area of the GC peak for acetaldehyde. This experiment was repeated for acrolein. The results obtained from standards prepared in this manner were used to determine acetaldehyde and acrolein in our monitor cigarettes. Agreement with those deliveries found by the collaborator (Table 3-4) was excellent.

In the procedure now in use for the determination of acetaldehyde and acrolein, a carefully selected monitor cigarette is smoked to calibrate the chromatograph, and the sample cigarettes are smoked. The areas of the peaks of interest are expressed relative to the corresponding peaks in the monitor chromatogram. The predetermined calibration factor, micrograms of acetaldehyde or acrolein determined colorimetrically per unit peak area, is used to compute the absolute delivery of the sample cigarette.

The major point of concern with this approach is that we employ a cigarette as our standard. Since the smoking and chromatographic analysis requires approximately 90 minutes, it is possible to run only one calibrating cigarette in any given day. It is obviously very important that a cigarette be carefully selected in order to obtain day to day reproducibility and meaningful results. Our experience is that reproducibility is approximately as good as found using chemical standards. We feel that the absolute results are more reliable using this approach than chemical standardization because of the inherently poor stability of the chemicals; with this procedure, the standards are taken precisely as are the samples.

Table 3-11 summarizes the precision of this determination in terms of per cigarette, per puff, and per gram of tobacco deliveries. The confidence interval for the second analysis is omitted because it involved only two observations.

3.22 Carbon Monoxide and Carbon Dioxide

The procedure in use for the determination of CO and CO_2 in the gas phase of smokes involves collecting the full gas phase delivery of a cigarette and a subsequent gas chromatographic analysis. The procedure and studies leading to its adoption were outlined in the past report [13] of this series. The method has been studied in collaborative tests and by an examination of the routinely generated data. A new column system has been adopted which doubles the number of chromatograms which can be generated in a given period of time.

An interlaboratory comparison of results for deliveries of carbon monoxide and carbon dioxide by selected cigarettes is included in Tables 3-3 and 3-4. Agreement is seen to be excellent in Comparative Study Number 2 (Table 3-4), acceptable for sample 23-S of Study Number 1 (Table 3-3), and poor for samples 21-S and 22-S of Study Number 1. The general trend is in agreement with expectations in that the collaborators in Study Number 2 employ a gas chromatographic procedure very similar to that used here. The collaborators in Study Number 1 employ a very different (on-line infrared spectroscopy) procedure. Interestingly, results reported [8] by the same technique for the 1R1 cigarette agree perfectly with that determined here (1.58 ml/puff CO by infrared versus 1.58 ml/puff by the

ORNL chromatographic method). Repeated analyses here of samples 21-S and 22-S produced the same results, within experimental error, as are given in Table 3-3. Additional studies would be necessary to resolve this inconsistency.

There are two possible disadvantages to the procedure in use here. First, the fact that single cigarettes are smoked at each port such that as few as four cigarettes contribute to the final result raises concern about how representative the result is of the complete batch of cigarettes. Agreement with collaborator number 2 and experience with other analyses involving small numbers of cigarettes suggest that proper cigarette selection greatly diminishes this danger. Second, the requirement of two separate chromatographic columns, one for the carbon monoxide determination and the other for carbon dioxide, increases the analysis time and requires specialty gas valving. Recent work, described below, eliminates this disadvantage.

A method for the determination of carbon monoxide and carbon dioxide on a single column has been described by Ohzeki and Kambara [26]. A similar column packing was prepared here from 42-60 mesh Amberlyst 15 ion exchange resin. The resin was washed with ethanol-water (90-10) and hydrochloric acid, then dried at 110°C overnight. The resin was washed again with ethanol and water, then treated with aqueous nickel (II) nitrate. The nickel form resin was then washed with water until no nickel could be found in the washings, then dried at 110°C for 8 hours. A 12 ft. x 1/4 in. stainless steel column was loaded with the treated resin and activated by heating to 225°C with hydrogen flowing through the column at a rate of approximately 150 ml per minute. The column was then operated at 80°C with a helium flow of about 50 ml per minute. When the column begins to lose its resolving power, it can be regenerated by heating to 225°C for two hours with a 150 ml per minute hydrogen purge. Figure 3-2 illustrates a typical CO-CO_2 chromatogram of the gas phase of a single cigarette. Table 3-12 summarizes typical results of CO-CO_2 analyses using the single column and the dual column (old procedure).

Table 3-13 summarizes the precision with which determinations of carbon monoxide and carbon dioxide may be carried out using both techniques.

The procedure, particularly that involving the single column system, is considered well suited to the responsibility of this program.

3.23 Formaldehyde

Formaldehyde continues to be determined using the chromotropic acid procedure described earlier [15]. Data are now available to better assess the reliability of the procedure. Formaldehyde values given in Table 3-4 illustrate excellent agreement with the collaborator. Good agreement was expected because the procedure in use here was adopted directly from the collaborating laboratory. Although collaborator number one did not provide data for formaldehyde from the special cigarettes, they report [6] that our values are consistent with their earlier experiences. Table 3-4 summarizes the precision with which the analyses have been carried out. It would appear the procedure is fully operational .

Concern was expressed earlier [13] that some formaldehyde might be lost due to inefficient trapping and/or its high reactivity. A pilot study was carried out in which the closed system designed for collecting NO_x samples was applied to formaldehyde trapping. Since the closed collection system contains the full delivery of a cigarette, losses should be minimized. Preliminary results indicated a higher delivery of formaldehyde using the closed (static) system than the normal open (dynamic) trapping system--15, 30, 37 μg/cig. for dynamic trapping versus 31, 45, 55 μg/cig. respectively for static trapping. The percentage increase was not constant for the three cigarette types studied as might be expected if system losses were responsible for the difference. Additional studies will be carried out if warranted.

3.24 Oxides of Nitrogen

Oxides of nitrogen are determined routinely as NO_2 using the Saltzman procedure [13, 31]. The previously described [13] sampling system continues to operate reliably. The single modification since the last report involved a machining of the filamatic pump syringe to further decrease the dead volume and subsequently reduce the fraction of the puff lost. Table 3-15 summarizes the precision with which this determination has been carried out.

Tables 3-3 and 3-4 include data for NO_x deliveries of the cigarettes studied. Agreement in Study Number 1 (Table 3-3) is seen to be very poor. This most likely results from the very great differences in analytical procedures--colorimetrically analyzed NO_2 in the gas phase trap at ORNL versus on-line spectroscopic analyses of fresh gas phase by the collaborator. Agreement in Study 2 (Table 3-4) is substantially better--both laboratories applied the Saltzman procedure. The results generated here are considered as reliable as possible within the limitations of the specified procedure.

Measurement of very fresh smokes for NO content is considered superior to trapping and subsequent analysis of NO_2. The colorimetric procedure itself, though very sensitive, is subject to interferences [31]. On-line spectroscopy is considered here to be the method of choice for determining the true NO content of smokes. The cost of establishing such a system here, in view of the relatively low importance of the parameter for present studies, is prohibitive. The use of gas chromatography coupled with a nitrogen-specific conductivity detector (presently available) shows great promise as a means of assaying fresh gas phase for NO. Preliminary observations demonstrate the ability to reproducibly visualize an NO response on fractions of a puff of smoke. A reliable standard is being obtained to allow careful quantitative calibration.

3.25 Hydrogen Cyanide

Hydrogen cyanide deliveries have been determined using the ascarite trapping-colorimetric measurement procedure described previously [13, 15]. Although the first analysis of the primary NCI experimental cigarettes proceeded well the second analysis, approximately nine months after the first, revealed several ambiguous results. Precision for given analyses was often very bad requiring that additional sets of samples be analyzed. Although agreement between analyses was reasonably good for most cigarette types, several cases were noted where significant differences existed. Other chemical parameters were in generally good agreement, thus suggesting that changes in cigarettes due to storage were not responsible for the apparently changed HCN deliveries.

Further examination demonstrated that substantially different results could be obtained simply by using different batches of ascarite. The

observation was independently confirmed by Collins et al [6, 7]. The practice of analyzing several batches of cigarettes to obtain a larger sample population and of using a given batch of ascarite for as many analyses as practical help to provide a generally reliable estimate of the relative deliveries of HCN. The practice is, however, both laborious and unsatisfying. A new procedure was sought to replace the questionable ascarite technique.

Collins et al [7] reported the use of silica gel as a trapping medium for HCN analyses. The procedure, optimized for automated analysis, was modified here to allow manual operations. Figure 3-3 illustrates the relationship between whole smoke hydrogen cyanide values determined using the new procedure and gas phase values obtained with the old procedure for the few cigarettes re-analyzed at this writing. The generally linear relationship is taken as evidence that the relative deliveries obtained on the gas phase earlier are applicable to correlative comparisons with gas phase bioassay results.

An additional important advantage of the new technique is that it is directly applicable to analyses of both gas phase and particulates. Whole smoke values may be more important because this is the matrix actually bioassayed. The whole smokes of selected experimental cigarettes are being analyzed for HCN with the new procedure to further test the reliability of earlier generated results.

Table 3-16 summarizes the precision obtained during the determination of HCN in gas phase by the ascarite procedure. Table 3-3 and 3-4 include data on gas phase deliveries of HCN obtained here and by the collaborators. Deliveries are seen to agree upon adoption of the new assay.

3.30 Condensates

Cigarette smoke condensates prepared at Meloy Laboratories [27] for mouse skin painting bioassay are submitted to ORNL for chemical study. Components routinely determined include nicotine, nicotine alkaloids, total phenolics, phenol, cresols, weak and very weak acids, phenanthrene, benz(a)anthracene, and benzo(a)pyrene. The results of the analyses are to be correlated with cigarette characteristics and bioassay results.

The NCI designed protocol specified that two individual batches of condensate, selected randomly from that prepared and submitted for bioassay, be analyzed for at least those components listed above. It was considered critically important that the analyses be carried out on samples actually bioassayed rather than specially prepared for analyses. Problems of sampling--insuring that the small aliquots received and analyzed were representative of the whole--became of overriding importance. Logistical problems--waiting until the final production runs to obtain many samples--resulted in considerable delays. A study of the data generated for the two primary samples of each type submitted for analysis revealed that differences between samples from a given cigarette could occasionally be much greater than could be accounted for by analytical error. These observations prompted a re-consideration of sampling protocols, sample scheduling, and analytical procedures. A third batch of samples, prepared in the same manner as were the bioassayed samples but specially for analysis, were submitted to obtain additional data. The results of a study of the differing sample treatment procedures were described in section 2.20 of this report. The added group of samples and detailed studies of the protocol greatly improve the confidence associated with the condensate analyses. Observations made during these added analyses and special studies will be considered in designing an improved protocol for future condensate analyses.

The single most important recommendation issuing from these studies is that as large a number of batches of condensate be analyzed as possible. Regardless of refinements in technique, examining only two or three batches allows too great an opportunity to obtain unrepresentative samples and allows any single error to contribute too heavily to the final result.

3.31 Dry Condensate Determination

Experimental study of the originally specified NCI protocol for condensate analyses suggests that this measurement may be second in importance only to sampling with regard to the reliability of chemical results on condensates. Condensate samples, received as approximately 50 wt % solids in 10:1 acetone:water, were sampled by aliquoting 100-200 mg of the

suspension into 3.5 cc septum-capped vials. Four such aliquots were contacted with dioxane containing a small volume of ethanol as internal standard, and gas chromatographically analyzed for water and acetone [13, 33]. The difference between the sample weight and water plus acetone weights is taken as the percent dry condensate. The critical nature of this determination becomes apparent when it is realized that this single value, obtained from four small aliquots, must be used to convert the results of all subsequent analyses to the "dry" basis.

This approach is subject to several criticisms:

1. A very small sample, approximately 100 mg dry condensate, is used. Losses of acetone during transfer can be variable and large percentage wise. Sampling is more difficult. The dry condensate composition of this small sample must be assumed representative of the much larger samples--including the 5-10 gram PAH samples.

2. Analytically--errors must be expected in (a) drawing the precise volume of dioxane, (b) ignoring the contribution of the condensate volume, and (c) the gas chromatographic measurement.

3. A method in which the result is obtained indirectly, by difference, is used.

An aggregate error due to the analytical procedure itself of five percent, a conservative estimate since gas chromatographic error alone for the internal standard technique can approach five percent, is equivalent to an error of 2.5 wt % in the dry condensate value for a 50 wt % suspension. Experience indicates that a 25 wt % suspension is substantially better for homogenization and sampling. Using this more dilute suspension will result in a greater, approximately 3.75 wt %, error from the analytical measurement alone. As the suspension becomes more dilute, improving sampling even more, the analytical error becomes very severe as you rapidly approach the point where the result is a small difference between two large numbers.

An alternative approach to determining the dry condensate composition of the suspension is to simply evaporate the water and acetone and weigh

the residue. Preliminary tests in which samples ranging from 100 mg to several grams were weighed into small vials and placed in a vacuum desiccator revealed that, given sufficient time, all would achieve a constant weight. The percent dry condensate was found to be independent of sample size and approximately the same as found from water-acetone determinations. The following general procedure was devised as a possible alternative sampling-dry condensate determination method:

(a) Condensate is diluted with acetone to provide a suspension of approximately 25 wt % solids.

(b) The suspension is transferred to a separatory funnel fitted with a side-arm, a mechanical stirrer, and Teflon stopcock.

(c) The suspension is vigorously stirred while withdrawing the amount required for the various analyses. Samples for most analyses, 100-500 mg dry material, are withdrawn into 3.5-30 cc volume capped vials. Samples for PAH analyses, 20-30 grams of suspension, 5-8 grams dry material, are withdrawn into 300 ml round bottom flasks fitted with a ball joint neck.

(d) The flasks containing the PAH aliquots are stoppered immediately and weighed.

(e) Approximately 25 ml of benzene is added to the flask and the flask is placed on a rotary evaporator.

(f) The contents of the flask are rotary evaporated at a reduced pressure of 15-30 cm Hg with the flask dipping slightly into a 37°C water bath.

The dry weights determined for the PAH aliquots are then used as determinations of the dry condensate composition for converting the results of the other analyses to the dry basis. This approach would appear to have several advantages over the water-acetone procedure:

1. The sampling technique allows the use of a more dilute, thus more homogeneous, sample. Large numbers of samples can be withdrawn quickly.

2. The large sample contributing to the dry weight determination minimizes weighing errors and errors due to solvent losses.

3. A direct method is used rather than a "by-difference" method.

4. The analysis is uncomplicated and requires a minimum of operator time.

5. Individual dry weights are generated for each PAH sample--the most difficult and among the most important analyses.

6. The procedure is immediately applicable to analyzing methanol suspensions--now being considered to supplant acetone for the chemical samples in view of its ability to provide better suspensions.

Table 3-17 summarizes typical results for percent dry condensate obtained using the gravimetric procedure. Aliquot to aliquot agreement is essentially the same as found using the gas chromatographic procedure. It can also be seen that relatively short periods of rotary evaporation are sufficient to remove the solvent. Much longer periods can be used without jeopardizing the results--evaporation time does not appear to be critical. Comparing results of the rotary evaporation of large samples with results of vacuum desiccating smaller samples to constant weight indicates (a) that sample size and method of evaporation, provided it is gentle, are not critical, and (b) that the dry condensate value obtained from the large samples is applicable to converting other results to the dry basis. Table 3-18 summarizes the results of an analyses of condensates dried by rotary evaporation for residual water and acetone. It can be seen that the residual water and acetone content is variable but does not exceed a total of 2%. One can expect that dry condensate values obtained gravimetrically should be higher by 1-2 wt % than those determined chromatographically.

Table 3-19 illustrates a comparison between dry condensate values obtained gravimetrically and chromatographically. It is seen that gravimetrically determined values are larger as was expected. Particularly disconcerting, however, is the occasional poor agreement between repetitive analysis when the chromatographic technique is used. Samples 2F and 6F agree well but the others, particularly 14F, are in poor agreement. Although most results have agreed acceptably in the course of past analyses when re-analyzed for dry composition, a single undetected grossly erroneous result affects the entire series of reported values for the corresponding run of the corresponding sample code. Repeated analyses gravimetrically are consistently in better agreement than those done chromatographically.

As demonstrated by the data in Table 3-20, the water/acetone procedure is basically capable of providing the proper result. Difficulties associated with the small samples and the susceptability of indirect methods to occasional gross error are most probably responsible for the occasional erratic result. Table 3-21 summarizes the precision with which water, acetone, and dry condensate values have been generated.

The gravimetric approach is considered preferable for obtainind dry condensate estimates to minimize the occurrence of erratic results. Both procedures will be applied to dry condensate determinations while final evaluations of the data are made.

3.32 Nicotine and Nicotine Alkaloids

It was reported previously [13] that the result obtained for nicotine alkaloids by steam distillation and spectroscopic measurement of condensate samples was consistently smaller than that found gas chromatographically. This is in contrast to expectations since the chromatographic method presumably detects only nicotine whereas the spectrophotometric method detects both nicotine and minor alkaloids. Table 3-22 summarizes final results for nicotine and nicotine alkaloids on given condensate samples--it is seen that the trend has continued throughout these studies. It must be noted, however, that the average results obtained by the two methods are often the same within experimental error.

Instances have occurred in which the nicotine result was substantially greater than the nicotine alkaloid result. A study of these analyses revealed that the nicotine standard had degraded to 90-95% purity. With freshly prepared (preparative scale gas chromatography) standard nicotine, the agreement between the two methods inevitably approached that typified in Table 3-23.

Since both procedures provide essentially the same result, either can be correlated with bioassay results to establish the nicotine alkaloid contribution to the biological effect of the condensate. The primary utility to using both procedures is as confirmation of the analytical result and, thereby sampling integrity.

Nicotine and phenol plus cresols analyses require final gas chromatographic measurement. Steps have recently been taken to improve the

efficiency of instrument usage and both analyses. An isothermal dual flame ionization, dual electrometer, Bendix 2600 gas chromatograph was acquired to allow both analyses to be carried out simultaneously and without requiring frequent column changing or instrument adjustment. Column and operating conditions were changed from those previously used to allow the two analyses to be carried out in the same oven. New conditions for nicotine are as follows: Column - 3' x 2 mm i.d., 10% Casterwax on 80-100 mesh Chromosorb G(HP). Column, inlet detector temperatures - 135°C, 200°C, 200°C. Carrier gas (N_2) flow rate - ∿ 125 ml/min. A chromatogram obtained under the new conditions for nicotine analyses is given in Figure 3-4.

Table 3-21 summarizes the precision with which nicotine and nicotine alkaloid analyses have been executed to date.

3.33 Phenolics

The phenolic content of condensates continue [13, 15] to be analyzed both colorimetrically and gas chromatographically. As is the case for nicotine and nicotine alkaloids, values obtained colorimetrically for total phenolics agree well with values for phenol plus cresols determined chromatographically (Table 3-22). A comparison of these results differs from a comparison of nicotine with nicotine alkaloids in that both phenol analyses are carried out on the same sample aliquots. The comparison is sampling-independent. Agreement between these values would not be expected to be better than the nicotine comparison because the procedures measure substantially different components.

The difficulties [13] with column stability and lifetime have been resolved by treating the solid support with 1% phosphoric acid prior to coating with (5%) Casterwax. Deterioration and poor reproducibility were apparently associated with loss of liquid phase and exposure of the acidic phenols to the neutral to slightly basic solid support.

As mentioned in the previous section (3.32), phenols are to be chromatographed in an instrument used simultaneously for nicotine analyses. The new column conditions to be employed are: Column - 12' x 2 mm i.d., 5% Casterwax on 80-100 mesh Chromosorb G(HP) pretreated with 1% H_3PO_4. Column inlet, and detector temperature - 135°C, 200°C, 200°C. Carrier gas (N_2)

flow rate - 40 ml/min. Figure 3-4 illustrates a chromatogram of phenol and cresols generated under these conditions.

Table 3-21 illustrates the precision with which the phenolic determinations have been executed. Considering both the precision and run to run reproducibility, results for o-cresol have been poorest. This is probably associated with the fact that the chromatographic peak for o-cresol was often so small as to be barely adequate for quantitative measurement. Attempts to increase the peak size by increasing instrument sensitivity have interfered with phenol and m-cresol plus p-cresol peaks. The new column conditions promise to improve this situation by providing a greater separation of the phenols from the solvent.

Interest in expanding the number of phenols routinely analyzed has continued on a low-priority basis. Since many other isomeric monohydric phenols are extracted along with phenol itself and the cresols, designing a suitable gas chromatographic column would be sufficient to allow determining other phenols with a minimum of added effort. Earlier studies [15] demonstrated that casterwax columns are incapable of sufficient resolution to make their use particularly valuable. Trixylol phosphate, however, allowed sufficient resolution but had not been studied here under isothermal conditions useful for routine analyses. Pilot studies have demonstrated that proper separating conditions can be achieved (Figure 3-5) but quantitative reproducibility was not tested. The recently reported [29] use of such columns for routine phenol analyses is encouraging.

3.34 Polycyclic Aromatic Hydrocarbons

The procedure followed for the routine determination of phenanthrene, benz(a)anthracene and benzo(a)pyrene is essentially the same as outlined previously [13, 15]. Primary developmental attention has been given to increasing recoveries of the polycyclics and to improving the efficiency with which the analyses can be carried out. Procedural modifications now in use are summarized below:

1. Sample is weighed into 300 ml round bottom evaporating flasks. Enough sample in the form of an acetone suspension is taken to provide 5-10 grams of dry condensate. After spiking with the

three carbon-14 labeled polycyclics, 25 ml of benzene is added to the flask and the flask is placed on a rotary evaporator. The sample is dried at a pressure of 15-30 cm Hg and bath temperature of 37°C until free liquid is no longer visible. A drying time of two hours is sufficient. The actually determined dry weight is used for the final calculation of results for the given aliquot.

2. The carbon-14 labeled compounds are diluted to a level such that milliliter aliquots can be used for spiking. This is faster than the use of 100 lambda micropipets and more accurate and reproducible than the Oxford pipet. A composite of the three labeled compounds is made up to 10 ml from which a 100 lambda volume is drawn and put into a 10 ml scintillator solution. This is the standard to which extraction recoveries are compared. A standard spike solution is prepared for each of the three compounds each time condensate spiking is performed.

3. The three minutes manual shaking used in the past has been found inadequate to insure complete extraction. The initial shaking is continued for a minimum of 30 minutes and the subsequent shakings for a minimum of 15 minutes. These minimum shaking periods are readily accomplished with no waiting time if two sets of condensates (8 sample aliquots) are extracted the same day.

4. The rate of extraction of phenanthrene by DMSO is low compared to BaA and BaP and three extraction stages are not sufficient for a satisfactory extraction. A fourth stage DMSO extraction has been added.

5. The concentrate from the second cyclohexane back-extraction (from DMSO-H_2O) is brought to 10 ml in benzene. A 100 lambda aliquot is counted, and the count is compared with the count of the standard prepared in 2. It has been found through counting efficiency studies that 60% uncorrected recovery at this stage is usually equivalent to 80% or better in actual

recovery. Further work on the sample is discontinued if the uncorrected recovery at this stage is less than 50%. True recoveries of better than 80% are consistently obtained up through the extraction steps.

6. The condensate from the florisil cleanup is made up only in hexane without the addition of 200 lambda of benzene as formerly. No loss of sample to the container wall was found. The danger of benzene prematurely deactivating the alumina column is thus avoided.

7. The initial volume of pure hexane used to elute the alumina column has been increased from 50 ml to 100 ml. This is followed by 120 ml of 8:1 hexane:benzene, 80 ml of 6:1 hexane:benzene, and 150 ml 4:1 hexane:benzene. This sequence provides cleaner subfractions--particularly for phenanthrene.

8. The difficulty in obtaining acetylated paper from commercial sources together with the inconsistent quality have prompted us to prepare our own acetylated paper. The experience to date has been very encouraging. The method reported by Spotswood [35, 36] is used with some modifications suggested by J. Bell [14].

Although a very large number of PAH determinations have been carried out in the past 18 months, few have been carried out which included all of the above modifications. The increased recovery brought about by the added extraction time and stage has been helpful in improving precision. An assessment of the combined effects of all modifications await completion of additional analyses.

Gas chromatographic methodologies used for the final measurement of phenanthrene and benz(a)anthracene have continued to receive attention. A column [4] of 8% Apiezon L on 80-90 mesh Gas Chrom Q in 18' x 1/8" o.d. thin wall stainless steel has been tested for verification of the values generated using Apiezon W and Apiezon H for phenanthrene and BaA determinations. Results from the various columns have been found to be the same within experimental error. In the course of these studies, however, it was noted that the Apiezon W columns provided phenanthrene peaks considerably more difficult to measure than did the Apiezon L column when the

phenanthrene subfraction was slightly less pure than normal. Isothermal operation required comparable separation times with the two columns. The Apiezon L column is now in use for routine phenanthrene determinations.

Apiezon H continues [13] to be used for BaA determinations. For an as yet undetermined reason, chromatographic peaks for BaA are frequently contaminated with a small impurity. Chromatograms on Apiezon L are no better than those on Apiezon H. This problem is under study.

Combined gas chromatography-mass spectroscopy has confirmed the identities of the phenanthrene and BaA peaks. The BaA peak, as normally visualized prior to the recent occurrence of the "impurity", was found to be at least 90% pure.

Final measurement of BaP, carried out by fluorescence, continues to provide reliable results. Ultraviolet measurement continues to be of interest as an alternate procedure. An advantage of the UV technique is that baseline absorption measurements can be conveniently used [4, 34] to assess the purity of the BaP isolate. As reported previously [13], gas chromatography can also be used for an independent BaP measurement.

The precision with which the polycyclic determinations have been carried out is summarized in Table 3-21. It is hoped that the increased recoveries will improve the precision as has been indicated in more recent results. The accuracy of the results will undoubtedly be improved by the increased recoveries.

Table 3-23 provides a comparison between the results obtained here and those obtained by collaborators employing a similar extraction technique but different liquid and gas chromatographic techniques. The collaborators results, generated in single aliquots from single lots of condensate, suffer greater than do the results generated here from the possibility of acquiring an unrepresentative sample. Although both the collaborators and the authors feel that agreement, particularly for phenanthrene, should be better, in the authors' opinion, agreement is generally good.

3.35 Weak Acids, Very Weak Acids, and pH

The procedures described [13, 15] in earlier reports for titrimetrically analyzing condensates for weak and very weak acids and for

directly measuring the pH of condensates have been used without modification. A useful finding in the course of these analyses is that the electrode for titrimetric acid determination can be rapidly brought to a usable state after long periods of standing by contacting the electrode with methanol-HCl.

The precision with which these assays have been executed is summarized in table 3-21.

3.40 Data Handling, Treatment, and Study

The data generated for each parameter during each analysis must be treated statistically to define the precision with which the result is generated. Subsequent analyses for the same parameter provide results which must be combined with earlier results--weighted averaging and statistically. Each individual determination contributing to the average result of that analyses must be examined statistically to locate outliers. For analyses of particulate matter and gas phase or whole smoke, results must be expressed in five different units and each must be statistically treated. Although each necessary operation involves straight-forward statistical techniques, the very large number of such operations required makes manual data-handling impossible. Considerable effort has been expended in making computerized data handling possible.

In the first stage of the treatment, data are entered on a per port or per aliquot basis and the computer executes rejection tests, computes the average result, the specified statistical definition of the result, and prints the information in format number 1 (Table 3-24). When the results of the first and second analyses are available, both presented to the computer and printed for record as above, the data are combined and presented in format number 2 (Table 3-24). For purposes of a final data report, now in preparation, the data are treated and presented in various other formats (Table 3-24). In addition to removing the enormous burden of manual computations, this approach minimizes the possibility of typographical errors inherent in manually prepared data reports.

An added advantage to computerized data handling is that all the data, in every set of units and including the per aliquot or per port

results of any given analysis, either are in or can easily be transferred to the computer memory. Sophisticated data studies become easily available.

The data generated in the past analyses, particularly for condensates where different sample preparation techniques were used, required study to locate suspicious results. Correlation coefficients and F-test results were generated to aid in this study. When it was found that differences between average results were often very great compared to the precision of any given analysis, a visual display of analyses to analyses agreement was sought. The product, illustrated for a comparison of analyses numbers one and two for nicotine in TPM, is illustrated in Figure Number 3-6. Trends are easily visualized and pairs in poor agreement stand out readily.

Formating for the final data report and approaches to more sophisticated data correlations are in progress. A summary of the approach used to arrive at formats number 1 and 2 has been prepared by Dr. M. T. Kelley of this Division who has accepted responsibility for the computerized data handling. Dr. Kelley's summary is given below:

" Basically, the computations involved in the preparation of the tables of data for the Tobacco Smoke Analysis Program are very simple. The complications come only in introducing enough flexibility to cover a considerable variation in the number of observations, variation in units and hence the number of decimal places, and to provide a statistically valid basis for the rejection of outlying observations, and for preferring one set of observations to another.

The programs for preparing these tables are written in FORTRAN IV for the DEC System 10. This time-sharing computer system is ideally suited for this sort of computation because of the ease with which programs can be modified and rerun and because the C.P.U. time is generally less than five seconds per table.

It was originally expected that the experimental data for the tables would be entered via teletype, but it proved to be better to punch the data on cards which could be read into the computer. This gave more opportunity for checking the data before entry, as well as reducing computer connect time.

In general, the data consist of a set of up to six measurements of a component of cigarette smoke. Each of these measurements represents the average of four cigarettes smoked in one port of the smoking machine. These analyses are accompanied by the average weights and numbers of puffs for each port as well. These data have been obtained for each of the 23 types of cigarettes. In some cases the analyses were repeated because there seemed to be reason to doubt the first set.

A second set of measurements (RUN II) was also made on almost all of the parameters. These measurements were in some cases made months apart, which is some justification for treating the two sets separately and not as a homogeneous group.

On each group, 2 to 6 measurements, an average was computed, then the variance, i.e., $V = (\Sigma\ (x - \bar{x})^2)/(N-1)$ and from this an estimate of the standard deviation of the mean of the N measurements $Sx = S/\sqrt{N} = \sqrt{V}/\sqrt{N} = \sqrt{V/N}$. This is the quantity reported in the tables as standard error (STD. ERR.). This value is then used to compute what we have called the 95% confidence interval (95% C.I.) by the relation C.I. = (T(N)*Sx*100)/Av., where T(N) is the Student t factor for N observations. This is the analogue of the relative standard deviation but includes the Student t factor to take account of the small number of observations. If N were large t = 1.96 or $\sim$ 2 hence the popular use of 2σ for the 95% C.I.. When N is small, t is considerably larger than 2 (T(N) = 12.7, when N = 2). After the initial calculation of these statistical quantities, the data are re-examined to determine whether there are "outliers" which should be rejected. Using an estimate of the standard deviation based on all of the measurements on all of the cigarettes, the widest deviation from the average is tested to see whether its ratio to the estimated standard deviation is greater than the "studentized" extreme deviate ratio and should be rejected. Although the program provides the possibility of selecting 10%, 5%, or 1% rejection criteria, the 10% values have been used in all of the calculated tables. This rejection criterion is not applied if the number of observations is three or less, and no more than one value in a set is rejected. It it is determined that one value should be rejected, that value is marked with an asterisk and new statistical values are calculated omitting that one.

Since, in general, the deviations of the measurements of weights and number of puffs are much less than those of the analyses, all rejections are based on the analytical figures. However the corresponding values in the "Puffs/Cig." and "MG/CIG." tables as well as in the derived tables are marked (*) and these values are also omitted from the calculations. The information for this rejection is carried in a computer word whose individual bits constitute a map of the location of the asterisks for each type of cigarette.

When a repeat set of measurements was made, the variances of the two sets are compared to see whether one set should be selected over the other or whether they should all be considered as a single group. This selection is based upon a table of F values (10% level). If the ratio of variances is greater than F, the set having the larger variance is rejected. If this set happens to be the initial set of measurements, the two sets are exchanged. The values for the "Initial Analyses" are then recalculated including the repeat analyses if they were not rejected. The inclusion of the repeat analyses is, of course, obvious from the number of analyses shown in the table.

In most cases, a second set of analyses was performed. The two sets are shown in a combined table as RUN I and RUN II. No attempt is made in this case to select one set over the other. The two sets are combined to give an average and a composite "STD. ERR." and "95% C.I." is shown. However, since the two runs were, at least in several cases, performed some months apart there would be good reason to expect that the two values would be different due to possible changes in the samples. Therefore, the variances of the two sets were combined to give a new "STD. ERR." which represents the overall precision of measurement, but not the deviation of the combined sets of data from both runs from their mean. Calculations of all of the two sets as a homogeneous group could in some cases give a larger "STD. ERR." and "95% C.I.". Perhaps it should be done this way.

The programs for producing the tables of data are as follows:

CIG20.F4	CIG24F4	CIG27D.F4
CIG20A.F4	CIG25D.F4	CIG28.F4.
CIG21.F4	CIG25G.F4	
CIG23.F4	CIG25T.F4	

CIG20F.4 and CIG20A.F4 read data from cards into a standard matrix format, which is stored as a binary file. CIG20.F4 is for data for smoke from individual ports of the smoking machine while the A version is for condensate data. CIG21.F4 will allow the entry of data into the standard matrix format from the teletype. CIG23.F4 will print out the data in a stored matrix while CIG24.F4 will allow editing of headings or data in a stored matrix. CIG25D.F4 is the general program for producing tables with statistical data for the individual runs. This program produces six tables from three sets of input. It is the longest of all the programs because it contains the rejection tests as well as the statistical computations. CIG25G.F4 is a special modification for gaseous components such as CO and CO_2, where analysis is reported in volume percent. CIG25T.F4 is a modification for tables for tar, which is calculated by difference (TPM - NICOTINE - WATER). CIG27D.F4 combines the output from CIG25D.F4 for RUN I and RUN II or combines similar output for condensate analyses. CIG28.F4 is a special program for printing the pH of individual puffs in tabular form.

The input data have been stored by the computer on DECTAPE in approximately 45 files (binary). The combined output tables (RUN I and RUN II) are stored in approximately 75 files (binary) and about 10 files for single runs are also stored (binary). The $\sim$ 270 tables which have been printed have been combined into 12 ASCII files which are likewise stored on DECTAPE.

Some study has been made of the possibility of ranking data from the combined output tables. The ranking is feasible and it is feasible to rank into groups based on the "STD. ERR.". However, no conclusion has been reached on the best method of reporting the rankings or using them for calculating correlations with other data."

4.00 ADOPTION AND DEVELOPMENT OF NEW ASSAYS

An important continuing responsibility of the chemical assay project is to adopt or, where necessary, to develop new analytical procedures which extend the number and types of chemical components capable of being routinely or semi-routinely determined. Intensive effort in this direction is expended only when the advisory group identifies a particular component or parameter which is particularly important for determination in routine or special-interest samples. The rather more basic analytical research into novel methodologies or for the measurement of chemical species of possible but undetermined biological importance proceeds only after the primary routine and development activities are fully staffed. Even the most basic work is, however, directed toward laying a foundation upon which routine application is practical. Series of samples undergoing bioassay studies are generally analyzed by the new procedures to provide pilot data upon which an assessment of the biological importance of the parameter or component can be made. This activity, though a very small part of the overall effort, has proven valuable for increasing our response to newly identified NCI needs and for significantly extending the classes of components capable of routine measurement.

An area that has been of continuing interest to this group is that of component profiling. Basically, this technique involves the generation of chromatograms--gas chromatograms, particularly--for carefully specified fractions of smokes from two or more cigarettes. The peaks in the chromatograms, corresponding to the various components of the analyzed sample, serve as measures of the quantities of the various components present. Thus, such chromatograms serve as profiles of the components making up the fraction or subfraction under study. By comparing the relative if not absolute peak areas with those produced by a standard cigarette, it is possible to assess the contribution of a large number of components to any characteristic of the smoke--flavor, biological impact, whatever. Once a relationship is established, the peak(s) can be positively identified and the components subjected to rigorous quantitative analysis. Such an approach embodies the promise of surveying the possible contribution of

many hundreds of chemical species to any given bioassay result rather than a select few.

4.10 Particulate Matter

Particulate matter is substantially easier to collect and can be generated using more carefully controlled smoking parameters than can smoke condensate. It therefore constitutes a better matrix for the determination of smoke components than does condensate. Although condensates must be analyzed when minor (say, less than 0.05 wt % TPM) components are of interest or when direct comparisons with bioassay material is needed, particulate matter remains useful for many analyses. The utility of such analyses would be further improved with experimental demonstration that quantities per mg of dry particulate matter are directly related to quantities per gram of dry condensate. This would demonstrate the utility of TPM analyses for correlations with bioassays involving condensates. Characterizations of particulate matter composition are obviously of importance for correlation with bioassay results involving whole smoke exposures, and for direct comparisons of the chemistry of smokes generated by two or more cigarette types.

4.11 Total Phenols

It became of interest to quantitatively assess the phenolic deliveries of a series of filter cigarettes. The method of Oakley [25], previously modified for use in condensate analyses, was adopted directly for particulate matter analyses. Total phenolics in particulate matter was included in collaborative study number 1 (Table 3-3)--agreement was considered good. The relationship between phenolics in particulate matter and phenolics in condensate remains to be established.

4.12 Free Fatty Acids

Free fatty acids in the 14-18 carbon range have been suggested [5] as possibly important in experimental tobacco carcinogenesis. Studies of the component profiling [13, 14] of TPM suggested that these acids could be easily determined in particulate matter. A summary of the basic procedure follows:

1. A direct method for analyzing fatty acids in the total particulate matter of cigarette smoke has been developed for the gas chromatograph. A 6 ft. x 1/4 in. glass column is packed with 4% OV-101 on 80-100 mesh chromosorb G. Samples are run at a temperature of 190°C with He as carrier gas at a flow of 70 cc per minute.

2. Four cigarettes are smoked onto a Cambridge filter. The filter is placed in a 30 cc, septum capped bottle. Ten cc of distilled pyridine is added and the samples are allowed to stand over night. The samples are then mixed well with a vortex mixer and centrifuged.

3. 400 µl of the pyridine extract is placed in a 4 cc septum capped bottle and 200 µl of Bis(trimethylsilyl)trifluoroacetomide plus 1% trimethylchlorosilane solution is added. After heating for 30 minutes at 65°C, the bottle is cooled to room temperature and 5 µl aliquots are injected directly onto the column. The first fatty acid peak (Figure 4-1) is palmitic. Oleic, linolenic, and linoleic acids are eluted in one peak and stearic acid last. Standard solutions are made to cover the range of fatty acids in the samples. The standards are dissolved in pyridine and the silyl reagent is added in the same proportion as for the samples.

Carbon-14, palmitic, oleic, linoleic, linolenic, and stearic acids were used to determine the recoveries of each acid from the filter pads. Recoveries were all essentially 100%. Standard addition experiments demonstrated the possibility of quantitatively recovering the added acids. A study of the precision with which the analyses could be carried out revealed coefficients of variation (7 ports, 4 cigarettes per port) for palmitic; oleic, linoleic, plus linolenic; stearic; and total free fatty acids of 2.7%, 1.8%, 5.4%, and 2.4% respectively.

The full first series of experimental cigarettes has been analyzed using the new procedure to provide pilot data for biological correlations. The procedure is being summarized in detail for publication.

4.13 Component Profiling

Particulate matter continues [13, 15] to be "profiled" (sec. 4.10) following conversion of the particulates to silyl derivatives without prior

chemical fractionation. Important observations since the previous report are that pyridine alone is sufficient for reproducible extraction and that sufficient sensitivity exists to make evaporative concentration unnecessary. Conditions essentially as given for free fatty acid determinations (sec. 4.12) are used to prepare TPM for profiling.

Figure 4-2 illustrates a profile from the TPM delivered by a commercial filter cigarette. The profile was obtained by chromatographing the silylated particulates on an 18 ft. by 1/8 in. stainless steel column packed with 3% OV-101 on 80-100 mesh Chromosorb G-HP. The carrier gas (N_2) was maintained at a flow rate of $\sim$ 10 ml/min. while the temperature was held at 70°C for six minutes, programmed to 250°C at 5°C/min. then held at 250°C for eight minutes. The numbered peaks are identified in Table 4-1.

Figure 4-3 illustrates profiles of TPM obtained with the 18 ft. column for several cigarette types. Comparing the 1R1 profile with those for commercial cigarettes reveals that the TPM is compositionally very similar. The notable exception is the presence of added humectants in the TPM from the commercial cigarettes. The particulates from the 1R1 cigarettes are also very similar compositionally to those from the low nicotine 1A1 cigarettes except, of course, for the greatly reduced nicotine peak. A peak emerging at the point where propylene glycol elutes is quite prominent in the 1A1 profile. There is question as to the identity of the peak in view of the fact that propylene glycol is not used in the preparation of the 1A1 cigarettes. Gross differences between smoking products--the presence of menthol (eluting just before glycerol) and little cigars versus cigarettes--are easily distinguished.

It has been reported [22] that two to four times as many components can be visualized using a comparable sample preparation technique and high resolution capillary columns. Much greater discrimination is, thus, possible. Interest here, however, has been to develop a screening technique to detect major compositional changes with minimum sophistication. Typical 6 ft. x 1/4 in. o.d. columns have been examined for routine screening. Figure 4-4 illustrates a TPM profile generated by a 6 ft. x 1/4 in. o.d. column packed with 3% OV-101 on 80-100 mesh Chromosorb G-HP. The carrier

gas (N_2) was maintained at a flow rate of 60 ml/min. while the column was held at 90°C for 7.5 minutes, programmed at 4°C/min. for 17.5 min. and 5°C/min. to 270°C. Several peaks are identified in table 4-1.

Advantages of the shorter columns are convenience of operation, higher practical temperature limits and higher practical sensitivity operation. The reproducibility with which relative composition can be assessed using the short columns is illustrated in Table 4-2. It must be noted that no special precautions were taken to generate the profiles and that four cigarettes are sufficient to achieve this reproducibility. Studies are now in progress to optimize conditions such that the smoke from as little as a single puff can be profiled.

Examples of practical applications of the technique at its present state of development are given in sections 5.20 and 5.40 of this report. Extension of the technique to higher resolution profiles and/or routine screening for biological correlations awaits the availability of on-line computer data processing. Experiences to date are being summarized in greater detail for publication.

Component profiling, whether it be of particulates, gas phase, or condensate subfractions, takes on an added dimension with the use of element selective detectors. Flame photometric detection provides a convenient means of surveying sulfur-containing compounds (sec.4.21). Recent work with the Coulson conductivity detector suggests much promise for routinely profiling nitrogenous smoke components (sec. 4.23).

4.20 Gas Phase

Gas phase, that portion of the smoke which passes through the standard Cambridge filter, is often analyzed to avoid the problems inherent in sampling whole smoke. Although losses of some components to the particulates trapped on the filter from previous puffs can be significant, it is assumed that such losses are relatively constant and therefore cancel when smokes from several products are to be compared. Because relative deliveries are of primary importance for correlations with the results of biological testing, gas phase values are applicable to such comparisons.

Component profiling of the gas phase composition has taken the form of studying chromatograms generated for acetaldehyde-acrolein determina-

tions and studying the utility of element selective detectors. Techniques for the quantitative determination of compounds not included in early protocols and for improved measurement of routinely determined compounds have received the primary attention.

4.21 Sulfur Containing Compounds

Considerable progress has been made toward the quantitative analysis of the most volatile sulfur compounds in the gas phase of cigarette smoke. The adsorptive effects of copper, stainless steel, glass and Teflon for sulfur compounds have been investigated. Porapak Q, Porapak P, Deactigel and a special silica column from Tracor, Inc., were tested to determine which gives the best separation of H_2S, COS, CS_2 and SO_2, and to determine which requires the least conditioning--is the least adsorptive.

The Tracor silica column, chosen for the determination of volatile sulfur compounds in the gas phase, is of 6 ft. x 1/8 in. o.d. F.E.P. It is operated at 25°C with a nitrogen flow of 15 ml/min. The flame photometric detector was operated at 125°C with 150 ml H_2/min. and 20 ml O_2/min. The smoking machine used for this determination was fabricated at this laboratory and is shown in Fig. 4-5.

Of all the materials of construction tested for reactivity with or adsorption of sulfur compounds, Teflon was the most satisfactory. Glass was next best followed by stainless steel and copper. The sample loop and the line connecting the gas sample valve to the G.C. were changed from stainless steel to Teflon resulting in a 100-fold increase in detector response to H_2S. We plan to change all connecting tubing that comes in contact with the gas phase to Teflon and we expect an even greater detector response.

Columns of Porapak Q, Porapak P, Deactigel and Tracor special silica gave satisfactory separation of COS, H_2S, CS_2 and SO_2. Porapak Q and Porapak P required temperature programming to elute CS_2 in a reasonable time. Deactigel is silica gel that is deactivated with chromic acid, and for sulfur analysis, is further deactivated by washing with dilute HCl. Deactigel separates COS, H_2S, CS_2 and SO_2 in a reasonable length of time and with good resolution. However, considerable conditioning is required before quantitative data can be obtained. The Tracor column is slightly

superior to the Deactigel column with respect to separation of and resolution of the sulfur compounds, and is much superior with respect to required conditioning times. A chromatogram of volatile sulfur compounds in the gas phase of a Kentucky Reference (1R1) Cigarette is shown in Figure 4-6. Quantitative data for several cigarettes are shown in Table 4-3.

Effect of Aging. A three-way Skinner solenoid valve was placed between the gas sample valve and the vacuum pump, and was wired parallel to the Skinner valve that controls the duration of the puff. This arrangement allows gas phase to be trapped in a static condition in the sample loop and connecting lines. Only that in the loop and in the dead volume space of the gas sample valve is injected into the G.C. The result of aging is shown in Figure 4-7. Losses could be due to several causes: chemical reaction with other compounds in the gas phase, e.g., oxygen, and adsorption on the stainless steel inner wall of the gas sample valve are two possibilities.

Profiling. Approximately a dozen sulfur compounds are seen when gas phase is injected onto Porapak Q or Porapak P columns and the column is temperature programmed to 200°C. Injecting gas phase onto a silicone OV-101 column and temperature programming shows the boiling point of the final compound eluted from the column to be approximately 175°C. A system comparable to that used by Groenen et al [12] was tried unsuccessfully to generate a higher resolution profile of sulfur compounds. The search will continue for a column system applicable to wide-range profiling of sulfurs in the gas phase.

Sensitivity. The sensitivity of this approach is sufficient to guarantee the detection of even minor quantities of sulfur containing species. Sensitivity has been found sufficient, in fact, to determine the relative sulfur composition at various points *during* a two-second puff. The sulfur delivery is seen to vary both from puff to puff and during the course of a given puff (Figure 4-8). The implication of this finding is that great care must be exercised to select a sampling system which does not provide artifactual results. Studies, mentioned above, of the rapid loss of given sulfur compounds to adsorption and/or reactivity further restrict the selection of smoking systems applicable to providing quantitatively

reliable results. Work now in progress, using inert surface materials and sample averaging (sec. 2.11), should provide the first truly reliable estimates of the sulfur content of fresh smokes. Preliminary results continue to suggest that popularly held concepts of the sulfur composition of smokes are grossly in error.

4.22 Isoprene

Isoprene has been included in the list of components to be assayed on a pilot basis to test its possible contribution to skin painting results. Although isoprene itself, a gas phase component, probably does not contribute to the biological effect found by skin painting condensates, it may function as an indicator of the terpenes present--terpenes may contribute to the biological response.

A "profiling" approach was used to estimate the isoprene delivery. The isoprene peak area (peak number 1, Figure 5-4) was simply measured and its area expressed relative to the isoprene peak from a reference cigarette generated the same day (see sec. 3.21). The relative peak areas can be converted to absolute isoprene deliveries by calibrating the response for the reference cigarette. Since relative deliveries are sufficient for comparison with the biological results, calibration has not yet been attempted. Preliminary comparison with the biological data suggests the possibility of a good correlation.

4.23 Nitrogen Containing Compounds

The Coulson electrolytic conductivity detector has been tested very recently as a means to quantitatively visualize nitrogen containing components of smokes. Preliminary results have been very encouraging. Figure 4-9 illustrates a chromatogram, "component profile", of nitrogen containing species in the gas phase of a single puff of smoke. Acetonitrile, acrylonitrile, pyridine, and several basic nitrogen components are readily visualized. Simpler amines, methyl-, ethyl-, dimethyl-, etc. do not appear to be present in sufficient quantities for detection in these small sample volumes. Studies of possible losses to reactivity and/or system adsorption must be carried out to ensure the absence of the low molecular weight amines.

Conductivity detection also promises to offer an alternative to on-line spectroscopy for a reliable measure of NO free of NO_2 in fresh smokes. Figure 4-10 illustrates a chromatogram of gas phase NO generated by a single puff of smoke. Preliminary results suggest that NO values obtained by this technique agree generally with those obtained by on-line spectroscopy. A better standard is required before reliable results can be generated.

Studies of the utility of the system for determinations of nicotine (for fresh whole smokes in inhalation chambers), nitrogen heterocyclics, and hydrogen cyanide are planned.

4.30 Condensates

Condensates continue to be of interest as long as skin painting is the primary bioassay and wherever trace components must be measured.

4.31 Free Fatty Acids

The technique developed for rapidly estimating free fatty acids in TPM (sec. 4.12) is equally applicable to analyses of condensate. Approximately 500 mg of condensate suspension in acetone (250 mg dry condensate) is weighed into a graduated 15 ml centrifuge tube, diluted to 10 ml with dry pyridine and allowed to stand overnight. The samples are then treated as described for particulate matter. Chromatograms generated by the condensate samples are identical to those generated by the particulates with the notable exception that the peak preceding the oleic, linoleic, plus linolenic acids peak is absent. Quantitative reproducibility is comparable to that found for particulate matter analysis.

4.32 Metals

In view of the great interest in toxic metals in the environment together with the availability of various facilities to do trace metal analysis at this Laboratory, the condensate samples were analyzed for certain toxic metals. These metals are cadmium, chromium, copper, mercury, nickel, and zinc. Activation analysis provides a means of analyzing for nearly every metal in the ppm range; however, the analysis can become troublesome if separations are required. Cadmium, chromium, and nickel

can be determined by a direct count of the irradiated sample after about three weeks cooling. This method suffers in precision compared to the results that can be obtained by other techniques, and this fact together with the time required to complete an analysis have made us decide to use this only as a supporting method and then only when necessary.

The atomic absorption analysis for mercury and cadmium is an important tool for studying the environmental contamination due to these metals. This is a proven technique with good accuracy and precision. Unfortunately, only the mercury unit was available but the cadmium unit has just recently come on stream. The condensate is wet ashed in nitric acid with some perchloric added at the latter stages before injection into the flameless AA unit. A standard is injected after each run using the same matrix to check the effects of the background. The results of the analysis of a condensate series is shown in Table 4-4.

Spark source mass spectrometry with isotopic dilution is a recently developed tool that is particularly suitable for this analysis. The sample is wet ashed and spiked with the appropriate isotopically enriched standards. The precision and accuracy are very good. The analysis of the same condensate series is shown in Table 4-4. It is to be noted that mercury is one of the metals that are not suitably determined with this technique.

The recent developments in x-ray fluorescence are promising in that they point the way to a possible non-destructive method which involves the minimum of sample preparation. This is particularly important because of the ready possibility of contamination which increases with each added sample preparation step. We have made a preliminary run on the monochromatic x-ray fluorescence unit being developed by C. Sparks of the Metals and Ceramics Division, and there seems to be a good possibility that a direct analysis of the condensate may be possible.

4.33 Dibenz(a,j)acridine

The analysis for dibenz(a,j)acridine (DBA) was investigated particularly with respect to its behavior in the analytical scheme used for the polyaromatics. It was found that DBA accompanies the polyaromatics throughout the extraction stage. It is, however, held tightly by the florisil

column as a very narrow band at the top of the column. It elutes from the alumina column much later than does BaP. It has a fairly high extinction coefficient in the ultraviolet spectrum. Its behavior in the extraction scheme plus its tight absorption to florisil suggests a possible analytical approach that may be more direct than that reported in the literature.

4.34 Organosulfurs

Several fractions from the alumina column used in the liquid chromatographic separation of phenanthrene, benz(a)anthracene, and benzo(a)pyrene from condensate were injected onto an OV-101 column. Sulfur compounds were detected in the phenanthrene subfraction. The estimated boiling range of these compounds was 175-215°C. A chromatogram is presented in Figure 4-11.

A blank was run to assess the possibility that the sulfur peaks originated from the sample preparation procedure--possible since DMSO is employed for extractions. No sulfur peaks could be detected in the blank. The results must continue to be considered preliminary until confirmatory data are available.

5.00 SPECIAL STUDIES

Special problems or special-interest experimental cigarettes often require attention in the course of a fiscal year. Such studies are carried out at the request of NCI without added support. A percentage of the effort normally given to analyses of the prime experimental cigarettes is redirected to expedite progress in the special-interest project.

5.10 Ammonium Sulfamate-Treated Cigarettes

Cigarettes prepared using ammonium sulfamate treated papers have been of interest for some time [1, 21]. Skin painting tests reveal that condensates from cigarettes treated with ammonium sulfamate behave differently than controls not so treated. The normal battery of analyses were requested in search of a possible chemical explanation for the difference. Tables 5-1 and 5-2 summarize the results of these analyses on cigarettes prepared elsewhere for the study. The results are in general agreement with those obtained in other studies [21].

No obviously beneficial changes in smoke composition were detected. Problems with questionable manufacturing techniques complicate a full interpretation of the data. The data are being reviewed to assess the possible utility of examining other components or of preparing new cigarettes for study.

A side product of this study is a preliminary survey of the chemical difference between whole condensates and hexane extracts of condensates. Hexane extracts are employed in some skin painting studies to reduce nicotine toxicity. Examining Table 5-2 reveals that the hexane extraction produces condensates lower in nicotine and titrimetric weak acids and higher in phenolics and polynuclear aromatic hydrocarbons.

5.20 Marijuana Cigarettes

Marijuana cigarettes prepared under NCI auspices at Meloy Laboratories are the subject of intensive investigation elsewhere. The normal battery of chemical assays, substituting cannabinoids for nicotine alkaloids, were

requested to provide additional data. The final data report, submitted to the NCI on April 4, 1972, is summarized in Table 5-3. Data on those components not included in the routine battery of tests, particularly those for inorganic components, must be considered preliminary. Larger samplings and additional analyses are necessary to set the confidence limits for each component. The significant difference between the results for polycyclic aromatics in Lot 1 and Lot 3, possibly associated with sampling, decreases the confidence with which these values are known.

Trends are easily identifiable. Most striking is the similarity, chemically, between the smokes from marijuana and tobacco. The similarity is further illustrated by a comparison of the gas phase chromatograms generated in the analysis of acetaldehyde and acrolein (Figure 5-1). Major differences in addition to the obvious substitution of cannabinoids for nicotine alkaloids, include the lowered NO_x delivery and increased polycyclic aromatic hydrocarbon delivery of marijuana. A comparison of TPM profiles (Figure 5-1) further illustrates overall differences. In terms of individual components, isoprene is perhaps the most dramatically different being much lower in marijuana smokes than smokes from tobacco cigarettes. This may be a particularly important observation in view of the general correlation (sec. 4.22) between lowered isoprene and lower biological response.

A meaningful assessment of the relative physiological effect of marijuana and tobacco cigarettes awaits the result of biological studies in progress elsewhere.

5.30 Pilot Filters

Selective filtration is being considered as a prime variable for future experimental cigarettes. The particulate matter and gas phase portion of the regular battery of analyses was applied to a test of a pilot preparation of the tentatively chosen filter-types. Phenolics in particulate matter were included in the battery of tests. Whole smoke titrimetric acids were determined as a possible measure of the effectiveness of acid-selective filters. Results indicate that a measure of gas phase acids may

be preferable. A method may also have to be adopted to allow an assessment of the effectiveness of alkaline-selective filters.

A report on the results of the primary battery of analyses is in preparation.

5.40 Dog Inhalation Systems

The Auerbach technique of dog cigarette smoke inhalation involves connecting the respiratory tract of the animal directly to the cigarette by way of a tracheotomy. Smoke is drawn from the cigarette on inhalation. A one-way valve positioned between the cigarette and tracheotomy tube prevents air from being forced back through the cigarette on exhalation. The technique has been successful in eliciting a measurable biological response but has been criticized as overly traumatic for the animals and incapable of strict interpretation with the present absence of a quantitative definition of dosimetry. It was felt that steps should be taken to eliminate or at least minimize these difficulties prior to initiation of the scheduled NCI bioassays.

Personal observations of the earlier employed exposure system and studies of past protocols led to the conclusion that quantitating dosimetry for the existing system was impractical. It was felt that the best means of defining exposure was to define the quantity of smoke offered to the animal and maintain that quantity at a level such that all would be inhaled. A variable quantity of smoke will be exhaled through the mouth and/or nose but this loss also occurred with the earlier employed system. Although dosimetry is defined by the quantity inhaled rather than retained, this is considered a substantial improvement over the original technique.

A smoke generation-exposure device designed by Dr. S. P. Battista of A. D. Little, Inc., and in use there for chicken trachea exposures appeared easily adaptable to dog inhalation. Dr. Battista constructed such a device and demonstrated its utility for providing metered quantities of smokes to dogs. Figure 5-2, courtesy of Dr. Battista, diagrams the system. Smoke is drawn into a syringe during the puff cycle and is exhausted from the syringe into a holding tube upon completion of the puff. The smoke is inhaled from the holding tube directly into the tracheotomy

during normal respiration. A one way flap valve prevents the smoke from being blown out of the tube during exhalation. The device appeared to be well suited to the application, inexpensive, and easily repaired in the event of failure.

Two questions remained: how the quantity of smoke offered by the machine compared to that puffed by the dogs in earlier experiments and how the quality of the smoke offered by the machine compared to that generated under standard ("human") conditions. Of these, the first question is both the most important and most difficult to answer. In the absence of lung deposition data, and there remains some question about the practicality of acquiring such data, it is necessary to estimate the quantity of smoke "puffed" from the cigarettes by the dogs. This quantity can then be compared to that offered the animals in the holding tube of the ADL device. Unfortunately, no data are available estimating the average puff volume, duration, or frequency accompanying "dog puffing". It is hoped that this data can be obtained later.

The question of the composition of the smoke relative to that delivered by "human" smoking parameters is more academic. It can be argued that "human" parameters are artificial at best and that, in view of the generally poor success of most tobacco smoke inhalation carcinogenesis bioassay attempts, an altered composition may prove beneficial. Although care must be exercised to avoid gross changes in compositions and the presence of biologically serious artifacts, it is true that considerable variation in smoke composition must be allowed at the present stage of inhalation bioassay development. Data collected at this stage is of primary importance for defining the characteristics of any given device. Data on the composition of exposure environments produced by other machines coupled with more detailed studies of the smoke compositions will eventually allow the identification of machines producing exposure environments deviating unacceptably from the norm.

Simulated Dog Puffing. A Harvard Respirator was used to smoke cigarettes through Cambridge Filters. Puff volumes and frequencies (piston displacement and cycle time) were chosen to cover a wide range of minute volumes that resulted in burn times approximating that observed in dog

smoking. It must be emphasized that there is no correlation between the total volume of a normally respiring dog and the "puff" parameters selected. Inhaled air comes from both the mouth and tracheotomy. No data is presently available defining the fraction of the total respired air entering via the tracheotomy.

The smoke trapped on the Cambridge Filter was analyzed for TPM, water, nicotine, and tar. The gas phase components were collected for analysis by exhausting the filtered air into appropriate traps. The gas phase was collected in saran bags for the gas chromatographic determination of carbon monoxide and carbon dioxide, and in a cold trap for the subsequent cryothermal gas chromatographic analysis of acetaldehyde and acrolein. Analytical conditions were as previously described [13]. Profiles of the particulate matter components were generated using the previously described trimethylsilylation technique.

Table 5-4 summarizes the results of the chemical analyses. The minute volumes (cc of air drawn through the cigarette per minute) calculated from the piston displacement and cycle time must be considered approximate since they are calculated from set points rather than measured and because the pressure drop of the cigarette is changing and can be limiting. Animal to animal and cigarette to cigarette variation under dog puffing conditions make precise measurements of the simulated puff parameters unnecessary. Small amounts of blowback during the exhaust cycle of the respirator, a phenomenon also noted during dog puffing, further complicates strict comparison with analytical smoking.

ADL Machine. Several features of the ADL machine are suspected to produce measurable changes in either the quantity or composition of the smoke offered to the animals. A large volume valve isolating the cigarette from the syringe and the syringe from the holding tube was suspected to produce losses. The fact that the syringe must be lubricated with silicone oil to allow free movement and that the holding tube is tygon suggest the possibility of selective removal of smoke constituents. It must be emphasized again that, in the absence of data on other inhalation devices, such changes may be acceptable. The primary question deals with the quantity of smoke offered the animals.

TPM, water, nicotine, tar, and phenolics (colorimetric) were determined on an input filter placed between the cigarette and valve and on an output filter placed between the valve and smoke transfer line leading to the holding tube. TPM, water, nicotine, tar, carbon monoxide, carbon dioxide, acetaldehyde and acrolein were determined at the syringe output (between the valve and smoke transfer line), holding tube output and on samples generated by analytical smoking machines. All smokes were generated using a 35 cc puff of two seconds duration. Puff frequency, in an attempt to produce more smoke per cigarette, was set at two rather than one per minute. The results of these analyses are summarized in Table 5-5. Particulate matter profiles were also generated (Figure 5-3).

A study of Table 5-5 reveals that the ADL machine generates smokes of the same tar-nicotine content as that generated by a standard analytical smoking machine. A considerable loss, approximately 20%, of TPM occurs during the exhaust cycle. A volume-reducing insert is being fabricated to minimize valve dead volumes and will be tested as soon as possible. Short stand times, 5-10 seconds, in the holding tube are accompanied by very slight additional losses. The losses from the holding tube are generally within experimental error for the small sample, 4-8 cigarettes, tested.

Additional information about the composition of the smokes may be obtained by examining gas phase and TPM profiles (Figures 5-3 and 5-4, Table 5-6). Absolute peak sizes cannot be compared because of week to week changes in instrument sensitivity. The relative peak sizes, peak heights or areas, provide a measure of the relative quantities of the compounds present and therefore a measure of smoke composition. Substantial differences in gas phase components are evident in comparing the analytical smoker with the syringe exhaust and holding tube exhause of the ADL device. This difference is colored by the fact that the analytical smoker routes fresh smoke to the analytical column on a puff by puff basis whereas samples from the ADL device had to be cold trapped prior to routing to the column. Differences between the syringe exhaust and holding tube exhaust are particularly evident for those components eluting after acrolein. A relatively greater amount of these components are present in the syringe exhaust.

The TPM profiles suggest that the composition of the particulate fraction of the smoke maintains its integrity regardless of the routing. The TPM loss is, apparently, nonspecific.

ADL Machine Versus Simulated Dog Puffing. A measure of the relative quantities of various smoke components produced by dog puffing and those withdrawn from the holding tube of the ADL device compared to those found using analytical smokes may be obtained by an examination of Table 5-7. Water (and therefore TPM) and carbon dioxide deliveries are considerably greater under dog puffing conditions than with either the ADL or analytical smoker. Surprisingly, with the exception of TPM, water, and carbon dioxide, the component ratios found by dog puffing agree almost as well and occasionally better with the ratios found for analytical smoking than those found using the ADL device. This suggests that the composition of the smoke delivered by dog puffing is not as foreign as would be expected. The TPM profiles also demonstrate the basic similarity between analytical and dog puffed smokes. Agreement in gas phase composition (Figure 5-4) is not as consistent. Agreement between the ADL and analytically generated smokes is expected to improve as the TPM loss is decreased.

Table 5-8 summarizes the chemical data for "dog puffed" smoke generated with intermittent puffing at a nominal minute volume of 1000 cc, analytically generated smoke, and the smoke withdrawn or exhausted from the ADL holding tube. The bottom line summarizes the quantity of smoke offered the animals by the ADL holding tube as a percentage of the quantity withdrawn from the cigarette by direct dog puffing.

The data for a 1000 cc minute volume are proposed as an estimate of the maximum quantity of smoke drawn by smoking dogs. The burn time, 1.5-2.0 minutes to reach 23 mm, is approximately equivalent, perhaps slightly shorter, than is observed during actual dog smoking. Secondly, flows of this magnitude are reasonable assuming the cigarette acts as the restrictor which specifies the mouth-tracheotomy split ratio. Third, flows much in excess of these produce noticeable strain on the cigarette.

Accepting the 1000 cc minute volume data as an estimate of dosage previously received, we note that the ADL exposure system is expected to provide approximately one half the dosage per cigarette in spite of operating at two puffs per minute.

TOBACCO SMOKE INHALATION BIOASSAY METHODOLOGY PROJECT

6.00 INTRODUCTION

The objective of the inhalation methodology project is to establish an improved chronic inhalation bioassay to test the carcinogenicity of tobacco smokes. As such, biological studies direct the overall project.

Primary attention is being given to approaches which promise to increase the quantity of smoke reaching the lower respiratory tract of the exposed animals. Of these, techniques for bypassing the nose have received initial study. Intratracheal cannulation is being considered from both theoretical and practical standpoints. Novel means of bypassing the nose, manipulating exposure protocols for nosebreathing animals, studies of preferred animal strains and species, and considerations of novel endpoints for the bioassay are receiving increasing attention.

The large variety and highly sophisticated nature of the problems encountered have necessitated a broad experimental approach. The following sections, only partially included in earlier reports in this series, have been prepared as a summary of the activities in progress on a limited scale up to September 1, 1972 and significantly expanded as of that date.

7.00 HARDWARE DEVELOPMENT AND CHEMICAL STUDIES

The responsibility of the chemical group participating in this project includes two primary activities, (a) development, fabrication, and supply of exposure hardware and, (b) direct chemical support of the biological studies.

7.10 Hardware Development

Smoking Machines. The intermittent exposure device described in the previous [13] report continues to be the primary system in use for basic studies. Several additional complete assemblies have been supplied to the biology team to expedite the consideration of novel exposure schemes, lung deposition characteristics, and techniques for reducing toxicity.

Two important modifications were introduced since the last report. The design of the exposure chamber side plates was modified to accept an improved version (described below) of the animal containment tubes. Secondly, the puff system was modified to allow a single pump to operate two exposure units. Two such double units (Figure 7-1), allowing the exposure of a total of forty hamsters, are presently being evaluated.

The Hamburg II (Heinr. Borgwaldt, Hamburg, Germany) continues to be used for special studies. The exposure portion of the device has been modified to accept the animal containment tubes developed here and to provide for a better smoke exhaust.

Emphasis is now being directed toward the selection of smoking machines best suited for chronic exposures. The intermittent exposure device is considered questionably suitable for such studies for two primary reasons. For one, smoke stand times of up to 30 seconds allow for possibly significant changes in chemical composition. Secondly, intermittent air-smoke cycles increase the possibility of serious breath-holding and a commensurate lowered dose.

The continuous smoke generation system described by Wagner and Sidbury [38] is presently considered the best device for chronic experimentation. One or more such systems will be constructed as soon as possible.

Animal Containment. The previously developed [13] container can be used for both nosebreathing and cannulated animals. With practice, the

animal can be placed in the container with ease. Adequate head restraint needed to position the animals nose in the smoke chamber is provided. The primary disadvantage of the container is that the animals forelimbs cannot be extended forward as in normal posture. This results in substantial struggling, especially when smoke is introduced in the chamber.

A new container (Figure 7-2a and b) which is characterized by a slanted front piece has been developed. The hamster forelimbs can thus be extended in a more comfortable fashion. This design should lessen injury due to struggling and provide a more regular respiratory pattern. An added advantage of the device is the ability to adjust the nose position relative to the chamber wall for hamsters of different sizes. We find the design equally suitable to containing rats by simply increasing the internal dimensions of the body tube.

Cannular Devices. A wide array of cannula-mouthpiece assemblies have been fabricated for testing. Cannulas of polyethylene, Teflon, stainless steel and an unspecified rubber-like material supplied by the NCI have been examined for adsorption of smoke components (sec. 7.23), damage to the animal during insertion, and tolerance after insertion. Various tip designs were included as an additional variable during study of stainless steel cannulas. Dimensions, length and outer diameter, were varied to establish tolerable limits which allowed a maximum inner diameter. Discussed more fully in section 8.00, the basic conclusion is that insertion poses little problem, length (point to which cannula tip extends in animal) is critical, and the greater the flexibility the better an inserted cannula is tolerated.

The mouthpiece approach has continued to be used as the means of holding the cannula in place. It also prevents the animal from chewing or crushing the cannula itself. The primary problem has been to devise a mouthpiece which (a) does not interfere in the insertion process, (b) effectively holds the cannula, and (c) does not damage the animal. The mouth-molded rubber mouthpiece [13] remains the most effective design. Externally molded and hand-shaped mouthpieces were developed and found no better than the original design. Mouthpieces made of heavy gauge copper wire, hinged to the cannula using quick-setting rubber, show considerable

promise. The completely open nature of the copper ring and the hinge effect make insertion easy and flexibility good. The primary disadvantage to date has been damage to the mouth.

7.20 Chemical Studies

Progress of a methodological nature is summarized below. Results of the application of the techniques are given in section 8.00.

7.21 Lung Deposition Methodology

Studies on the production of radiolabeled cigarettes to be used in smoke deposition studies have continued. Tests were conducted to see which of four procedures would be most effective in uniformly labeling cigarettes with ^{14}C-hexadecane. These procedures were butt-end syringe-injection, hand injection of the whole cigarette, machine injection of the whole cigarette (up to butt), and spraying loose tobacco followed by rolling the cigarettes using a "Laredo" cigarette maker. Precision was about the same for all four procedures. Because of the inconvenience of having to roll relatively large numbers of cigarettes for deposition and other studies, the machine injection method was chosen for further studies.

A puff by puff study showed that the activity per mg of particulate matter was constant within about 10% (rsd) when either ^{14}C-hexadecane or ^{14}C-dotriacontane is used as the label. Reproducibility of labeling from cigarette to cigarette is about 5%. There was no decrease in the activity of ^{14}C-dotriacontane labeled cigarettes when they were stored for at least 25 days, but about 50% of the ^{14}C-hexadecane label was lost after seven days storage. Analysis of the smoke from both types of labeled cigarettes indicated that about 20% of the dotriacontane and 40% of the hexadecane go into the mainstream smoke while about 0.5% and 0.2% respectively pass through a Cambridge filter and are found in the gas phase. The convenience of preparing cigarettes well in advance of their use and the fact that dotriacontane is an actual smoke constituent resulted in the selection of dotriacontane as our primary label.

The procedure for the determination of the ^{14}C label in the body tissues of hamsters mentioned in the previous report in this series has

been modified. The tissue is dissolved in ethanolic-potassium hydroxide as described, but instead of the label being extracted into petroleum ether, an aliquot of the solution is treated with hydrogen peroxide and is then counted directly in a toluene scintillator solution. Blood can be processed in the same manner as the tissue. Small samples such as the larynx, trachea and esophagus can be counted directly in the scintillator solution without prior treatment. Recovery of both hexadecane and dotriacontane averages about 90% from the various organs, while reproducibility is about 5%.

Several experiments were carried out to study the residence time of the two labeled hydrocarbons in the various organs of hamsters. The label was introduced intratracheally in a gelatin solution. Results indicated that over a six hour period, there was very little loss of dotriacontane from the specific organs or from the sum of the body parts which were analyzed. The loss from the lungs as well as from the total was about 5%. On the other hand, there was a loss of about 50% of the hexadecane from the total body parts analysis and a loss of about 65% from the lungs. This was taken as added evidence that dotriacontane is the superior smoke label.

A total of 25 smoke deposition experiments have now been completed with hamsters as the test animals and using both ^{14}C-hexadecane and ^{14}C-dotriacontane as the labels. One series of tests involved filtering the smoke and allowing the animals to breathe the gas phase only. With dotriacontane about 0.015% of the estimated input of label was found in each animal while for hexadecane the figure was 0.03%. With both cannulated and non-cannulated animals, about 50% of the hexadecane activity was found in liver and blood, while about 70% of the dotriacontane activity was found in the liver. The blood was not analyzed in this case. Slightly more dotriacontane was found in the lungs of cannulated animals than noncannulated, while the reverse was true of the hexadecane activity. Of primary importance, the gas phase contribution to the counts found following whole smoke exposure can be expected to be undetectable.

A series of tests comparing cannulated with nosebreathing hamsters which were allowed essentially no clearance time indicated that more than

90% of the dotriacontane activity in unfiltered smoke deposits in the lungs of cannulated hamsters compared to around 50% for nosebreathing animals. With the latter animals about 40% of the activity is found in the head and 10% in the stomach. When the animals are allowed a short time (< 1 hour) for clearance, the 90% figure holds true for the lungs of the cannulated hamsters, but for the nosebreathing hamsters the figures are closer to lungs-60%, head-10%, and stomach-30%. Thus, to obtain values for the initial regional deposition of particulates, it is necessary to hold exposure and sacrifice times to an absolute minimum. Longer exposure times--whole cigarette rather than single puff exposures--are applicable for determinations of total body and lung doses.

7.22 Nasal Filtration

Studies are under way to examine the feasibility of determining qualitatively and quantitatively, the absorption of cigarette smoke components by the nasal system of hamsters. The general procedure to be followed is to insert a tube into the hamster's trachea in order to draw the smoke through the nasal passages, or alternatively, through a bypass cannula as a control measure. A second tracheal insert allows the animal to breathe and maintain normal circulation while anesthetized. The desired phase of the smoke is trapped and examined by gas chromatography. In the single test carried out so far, 10 ml puffs of undiluted smoke were drawn through the test animals and the whole smoke analyzed for acetaldehyde and acrolein. Approximately 50% of each of these compounds were apparently absorbed by the hamster's nasal system under the test conditions.

In order to facilitate future tests in this area, a small smoke chamber has been fabricated, and Cambridge filter holder have been adapted to hold 13 mm diameter filters for trapping small amounts of particulate matter. Pilot studies have demonstrated the possibility of quantitatively determining nicotine and generating component profiles on these small samples.

7.23 Deposition in the Cannula

Since the purpose of cannulation is to avoid trapping of particulates by the nasal turbinates, it is obviously important to select a material

which does not itself trap particulates to construct cannulas. An estimate of the relative affinitys of polyethylene, Teflon, stainless steel, and an NCI-provided material for particulates was sought. Cannulas of the four materials were constructed such as to maintain inner diameter, outer diameter, and length as equivalent as possible. The cannula under study was then positioned in the exposure chamber as it would be if a cannulated animal was present. The external end was attached via a Cambridge filter to a Harvard respirator pump and respirated with 1 cc volumes 117 times per minute while labeled cigarette smoke was puffed into the chamber. The respirator was plumbed such as to exhaust the filtered smoke back through the filter and into the chamber. The quantities of ^{14}C-hexadecane and ^{14}C-dotriacontane on the filter pad and in the cannula were then determined.

The results, expressed as percent of the total (filter plus cannula) activity in the cannula were as follows: for hexadecane--polyethylene= 29.5%, Teflon=4.8%, stainless steel=0.9%, and NCI=33.0%; for dotriacontane--5.4%, 8.3%, 0.9%, and 9.4% respectively. It can be seen that from the standpoint of losses of particulates, stainless steel is the preferred cannula material.

It is important to note the very different results obtained when dotriacontane is used rather than hexadecane. These data emphasize the tenuous nature of extrapolating from the label to weight of particulate matter. It also suggests that components differing only in volatility must be expected to be removed to different extents by the cannula.

7.24 Benzo(a)pyrene in Tissues

A procedure for the determination of Benzo(a)pyrene (BaP) in mammalian tissue has been developed. The tissue samples are dissolved in an ethanolic-potassium hydroxide solution, and these sample solutions are then extracted with petroleum ether. The determination of BaP in the extract is made by fluorescent spectrophotometry using an excitation wavelength of 368 nm and an analyzer wavelength of 409 nm. As little as 0.1 μg of BaP can be extracted and determined; however, because of tissue background fluorescence, results tend to be erratic with quantities of

BaP less than 0.5 μg. Ultraviolet spectrophotometry at 298 nm or visible spectrophotometry at 298 nm or visible spectrophotometry at 385 nm can be used with quantities of more than 10 μg of BaP, although a persistent very fine turbidity in some tissue samples limits the use of the former. This development work has been done on surrogate samples prepared by adding BaP to previously digested samples of lungs, heads, and stomachs. Recovery of BaP is about 90% with a precision of about 5%. This procedure has been successfully applied to a series of hamster tracheas that had been injected with a suspension of BaP in gelatin.

8.00 BIOLOGICAL STUDIES

It is well documented that certain cigarette smoke components are carcinogenic when instilled in the respiratory tract of rodents [3, 18, 30, 37]. However, most inhalation exposure experiments designed to induce cancer have been unsuccessful. The work of Dontenwill et al [10] and our own unpublished observations show that nasal filtration of components of cigarette smoke is one likely explanation for the lack of a carcinogenic response. In addition, cigarette smoke, relative to its acute toxic effects in rodents, has a low carcinogenicity. Toxicity thus limits the smoke exposure animals can tolerate. Therefore, the most likely reason that so many experiments have failed to induce respiratory tract cancers by smoke inhalation is due to inadequate dosage.

The Biology and Analytical Chemistry Groups are working jointly to test the effectiveness of nose bypass devices for increasing lung deposition. Alternate methodology is being designed to maximize smoke exposure and minimize carbon monoxide and nicotine toxicity in nosebreathing animals using smoke exposure schemes that reduce buildup of toxic components. Another phase of the program is concerned with selecting the strain and/or species of laboratory animals with low susceptibility to the toxic effects and high susceptibility to the carcinogenic actions of tobacco smoke. A fourth area of research centers around the detection of preneoplastic and early neoplastic lesions in smoke-exposed animals using exfoliative cytology.

8.10 Nosebreathing Versus Cannulated Hamsters

A comparison of lung deposition in nosebreathing and cannulated animals has been made by exposing hamsters to cigarette smoke containing the carbon-14 n-alkanes hexadecane (C_{16}) and dotriacontane (C_{32}). The first series of experiments were designed to test the influence of the label and the labeling technique on the dose of radiolabeled smoke deposited in the respiratory tract of nosebreathing and cannulated animals, and to test the effectiveness of the cannula as a nose bypass device. Table 8-1 shows the results of our first experiment. The animals were exposed to smoke of

a whole cigarette labeled with ^{14}C-hexadecane either in the butt end or distributed throughout the cigarette. ^{14}C-hexadecane is deposited in the lungs and measured. Location of the label in the cigarette (butt end or throughout) causes virtually no change in deposition either in the nosebreathing or cannulated hamsters. The cannulated animals, however, have twice as much lung deposition as the nosebreathers.

In another experiment, nosebreathing and cannulated hamsters were exposed to the smoke of a whole cigarette with ^{14}C-dotriacontane distributed throughout the cigarette (Table 8-2). Exposure to dotriacontane-labeled cigarettes results in substantially increased lung deposition in both nosebreathing and cannulated animals over exposure to hexadecane-labeled cigarettes. Cannulated animals in these experiments show approximately a 50% increase in lung deposition over nosebreathers.

Several experiments are summarized in Table 8-3 in which nosebreathing and cannulated hamsters are exposed to a single puff of ^{14}C-dotriacontane labeled smoke and sacrificed immediately after a 30 second exposure by cervical spinal cord separation. Deposition of label in the lungs, organs of the upper respiratory tract and the G.I. tract is shown. The cannulated animals show an increase in lung deposition 2.7 times that seen in nosebreathers. Approximately half of the total body label in nosebreathers is deposited in the lungs, while the rest is deposited in the head (nasal turbinates) and gut. In cannulated animals, virtually all of the total body label is found in the lungs. The data suggest that the cannula allows these counts normally deposited in the head and gut of nosebreathers to appear in the lungs. The effect of the cannula, however, appears to involve more than a shifting of head and gut counts to the lungs, since the total body counts in cannulated animals is also increased (1.54 times nosebreather).

Cannulated hamsters were observed to breathe at a lower rate than nosebreathers and it was suspected that changes in respiratory parameters might further explain the increased lung deposition. The respiratory parameters (tidal volume, respiratory rate, minute volume) of nosebreathing and cannulated hamsters were compared (Table 8-4). The technique for these measurements was similar to that described by Amdur and Mead [2]. Changes

by the hamster in respiratory rate and air displacement (tidal volume) in a plethysmograph were detected by an attached pressure transducer. Impulses from the transducer were fed directly into a DR-8 electronic recorder (Electronics for Medicine) which had previously been calibrated to convert such impulses directly to tidal volume (cc) and respirations per minute.

The values for tidal volume breaths/minute and minute volume of nosebreathing hamsters are within the range of those which have been reported by Guyton [16]. Drastic changes in tidal volume and respiratory rate are seen, however, in cannulated hamsters. The cannula causes a 68% increase in tidal volume accompanied by a 61% decrease in respiratory rate. The minute volume is approximately the same in both nosebreathers and cannulated animals. This means that, during a given interval, the total volume of air being respired is nearly equal for both groups. However, the deep, slow breathing pattern of cannulated animals favors deposition of particles in the deep lung tissues. In relatively rapid and shallow breathing, air movement is greatest in the upper portion of the respiratory tract; therefore, deposition of particles in this area is favored. From these observations, it seems reasonable to propose that the increased deposition seen in cannulated hamsters is due to a combination of shifting head and gut counts to the lungs, and an increase in tidal volume favoring deep lung deposition.

An experiment was designed to test the influence of changes in tidal volume on lung deposition. The hamster's respiratory muscles were paralyzed with curare (1 μg/gram body wt.). The animals were then placed in an airtight chamber so that the respiratory parameters, tidal volume, and respiratory rate could be controlled artificially with the aid of a respirator. All animals were cannulated so that respiratory geometry (i.e. nasal filtration vs. cannula) would not be an influencing factor. Respiratory parameters simulating those seen normally in cannulated animals were imposed on one group, while parameters simulating nosebreathers were imposed on a second group (Table 8-5). All animals were exposed to a single puff of a dotriacontane-labeled cigarette for 30 seconds, then sacrificed immediately by cervical separation of the spinal cord. Animals having a

relatively high tidal volume and slow respiratory rate (group I) show a two-fold increase in deposition over animals with low tidal volume and fast respiratory rate (group II). This experiment supports the idea that much of the increased deposition seen with the cannula is due to increased tidal volume.

The deposition experiments clearly show that substantial trapping of labeled particulates of cigarette smoke occurs in the nose, and that this can be prevented by the cannula. A test for nasal filtration of gas phase components was performed to determine if the cannula would increase the amount of these components reaching the lower respiratory tract. The procedure requires surgical alteration of the respiratory tract; the trachea is exposed and severed, and a polyethylene cannula inserted into the distal half to allow the animal to breathe. Smoke is drawn through the nostrils and passes into a stainless steel cannula (inserted into the larynx and proximal half of the trachea) and then through a Cambridge filter into an attached gas syringe. For the control, smoke is drawn directly through the cannula into the Cambridge filter and gas collecting syringe. The gas phase collected in the syringe is then injected into a gas chromatograph for analysis.

Gas phase profiles of cigarette smoke passing through the control device only and through the hamster's nose are compared in Figure 8-1. The peaks corresponding to isoprene, acetaldehyde and acrolein appear significantly decreased by nose filtration. These changes were quantitated for acetaldehyde and acrolein and both appear to be decreased approximately 50% by the nose (Table 8-6). These observations suggest that bypassing the nose will increase gas phase as well as particulate deposition of cigarette smoke.

The feasibility of using the laryngeal-tracheal cannula in chronic studies is being pursued. Brief, daily insertion of the cannula just past the larynx has been performed on one group of hamsters for five months without any noticeable gross damage to the animal. Metofane gas anesthesia is used to facilitate cannulation. Mild squamous cell metaplasia of the tracheal epithelium was noted histologically. Maintaining cannulated animals for more than several hours each day, however, has resulted in high

mortality. The animals tolerate the cannula fairly well up to two hours before they begin to die. Death in these animals is generally due to damage to the larynx and trachea which results in hemorrhaging and plugging of the new cannula. A low percentage of cannulated animals survive four to five hours daily cannulation for several days but die later apparently from asphyxiation due to laryngeal and vocal cord edema causing closure of the air passage.

Silicone rubber tubing is currently being examined for use as a cannula. This material is extremely flexible and it is hoped, will reduce the traumatic damage seen with the current (polyethylene) cannula.

8.20 Permanent Tracheostomy

The progress thus far with the cannula shows that the principle of nose bypass is valid in increasing lung deposition of cigarette smoke components. Since the possibility exists that the cannula may prove too traumatic for chronic studies, tracheostomy is being explored as an alternate means of bypassing the nose.

A permanent tracheostomy offers distinct advantages over cannulation since only one or two surgical operations and a similar number of anesthesias would be required during the animal's lifetime. The animal could be allowed to breathe through the tracheostomy only during smoke exposure; thereafter, the tracheostomy would be occluded to prevent food particles and other air contaminates from entering the respiratory tract.

Two tracheostomy designs are currently being tested (Figure 8-2). Polyethylene and medical grade silicone rubber tubing (Dow Corning) are being evaluated as potential tracheostomy tubes. In the first design (Figure 8-2a), a piece of tubing with flanged edges is inserted through a circumscribed opening on the ventral tracheal surface. The edges of the tubing are then sutured to the ventral surface of the trachea and the wound closed with the tubing protruding through the skin. Animals with this type tracheostomy survive two to three weeks before dying from congestion and pneumonia caused by the cannula blocking the flow of mucus. The more recent design (Figure 8-2b) has the tubing sutured to the outside of the trachea, to avoid the problem of blockage of mucus and air flow.

Two surgical operations are required. The tubing is first sutured to the outer tracheal surface and the wound is closed. Two weeks later a trocar is inserted through the tubing into the tracheal lumen to establish connection between the two.

8.30 Maximizing Exposure Schemes for Nosebreathing Animals

Tobacco smoke is a complex mixture of many toxic substances, most of which are acute in nature. Carbon monoxide and nicotine are the two most toxic components which usually prevent exposing rodents to high doses of cigarette smoke. Attempts are being made to reduce overall cigarette toxicity by exposure schemes which first of all do not favor buildup of toxins to harmful levels, and secondly, allow sufficient time between exposures for toxins to decrease to tolerable levels.

A considerable number of studies have been made on carbon monoxide toxicity to determine how fast CO-Hb increases in the blood in response to inhalation of varying quantities of smoke. One study is shown in Table 8-7 in which hamsters were exposed to varying numbers of cigarettes in the intermittent ORNL smoking machine. The CO-Hb level was nearly 50% after 50 minutes (10 cigarettes) of smoke exposure.

Other studies were designed to determine the rate of removal of CO-Hb from the blood after levels of 40-50% were reached either by inhalation of cigarette smoke or by inhalation of pure carbon monoxide. A rapid decrease of CO-Hb occurred during the first hour when the animals were allowed to breathe air. Administration of oxygen, rather than air, considerably enhanced the rate of CO-Hb removal.

These type experiments led to several chronic smoke exposure schemes which would prevent rapid buildup of CO-Hb levels and would allow for elimination of CO-Hb. This was accomplished by allowing 15 minute air breaks between exposure to two to three cigarettes, and also by allowing at least one, two-to-three-hour break after exposure to four to five cigarettes.

Some examples of the chronic exposure schemes which are being tested are as follows: Intermittent (30 second smoke, 30 second air) machine set at 10% smoke, continuous machine set at $\sim$ 7% smoke.

1. 40 minutes of smoke (8 cigs./day) intermittent smoker

   2 cigs., 15 mins. air; 2 cigs., 2 hrs. air; 2 cigs., 15 mins. air; 2 cigs.

2. 50 minutes of smoke (10 cigs./day) intermittent smoker

   2 cigs., 15 mins. air; 3 cigs., 2 hrs. air; 2 cigs., 15 mins. air; 3 cigs.

3. 40 minutes smoke (4 30-cig. cycles) continuous smoker

   4 10-minute smoke exposures per day with a 2-hour air break between each 10 mins. smoke exposure.

All chronic exposure experiments have sham smoked and shelf controls; periodic CO-Hb assays are performed on all animals.

The results thus far, show that animals can be exposed to at least 10 cigs./day (50 mins. of smoke) in the intermittent machine, and possibly more. This suggests that with appropriate smoking schemes, we may be able to give much higher smoke exposure than has been reported possible by other investigators.

8.40 Rodent Strain and Species Selection

Several factors explain why the hamster is thought to be the rodent of choice in smoke inhalation carcinogenesis studies. Bronchiogenic carcinomas can be induced in hamsters via topically applied chemical carcinogens. The hamster, in contrast to the rat and mouse, is quite resistant to respiratory infections and therefore, is more suitable for chronic studies involving the irritant cigarette smoke. In addition, the hamster is more resistant to the acute toxic effects of cigarette smoke, especially nicotine, and is able to tolerate larger doses of smoke.

One possible disadvantage of using the hamster is that its susceptibility to the carcinogenic effects of PCH is considerably less than that of the rat [23]. Therefore, one important part of this project is to identify the type of animal that has relatively high susceptibility to tobacco smoke carcinogenicity and relatively lowest susceptibility to smoke toxicity.

Within the hamster species, different strains are involved in acute toxicity studies. A total of six strains are available; five are in-bred:

(a) LSH/SSLAK, (b) PD 4/SSLAK, (c) LHC/LAK, (d) MHA/SSLAK, (e) 87.20 from TELACO Bio. The sixth strain, which has been most involved in smoke studies today is strain RGH/ARS--Sprague Dawley. Preliminary acute toxicity studies suggest that strain 87.20 may be considerably more resistant to acute toxicity than the Sprague Dawley strain.

Another group of experiments have recently been started to expose rats to cigarette smoke. The rat is more susceptible to nicotine toxicity than the hamster, and is also more susceptible to respiratory infections. However, preliminary experiments indicate acute toxicity can be somewhat circumvented (especially on the intermittent smoker) by not allowing nicotine to increase to lethal levels, and by allowing time between exposure for nicotine to be metabolized in the body. A specific pathogen-free rat colony is available which has been free of respiratory disease for several years. By working with these animals in the confines of a barrier, we hope to prevent respiratory infections which have been seen in other laboratories.

We are encouraged to proceed with rat studies because of recent studies which showed tobacco smoke condensate of one cigarette implanted in the lungs of rats resulted in a high incidence of squamous cell carcinoma within a year to year and a half [37]. This observation points out that rats are highly susceptible to tobacco smoke carcinogens.

8.50 Cytology

A method has been developed for collecting exfoliating cells from the lungs of hamsters [32]. Studies from chemically induced cancers show that precancerous lesions can be detected several months before animals die from invasive carcinoma.

Cytology samples are being taken periodically on animals involved in chronic smoking experiments. Early results are encouraging in that metaplasia and moderate cellular atypia were detected in several animals which had been exposed to 10 cigarettes per day (50 mins. smoke) for five months.

It is hoped that sampling of exfoliating respiratory tract cells will be expanded as larger scale smoke inhalation studies are initiated. Ideally, this technique will allow detection of precancerous lesions in smoked

animals and lead to an earlier diagnosis of cancer. If the diagnosis time can be decreased by even several months, the economics of bioassay experiments would be greatly improved.

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TABLES

Section 2:00

Table 2-1 Comparison of Puff Numbers from the Standard Four-Port Machine and Six-Port Sequential Smoker.

Table 2-2 (same page as 2-1)
Chromatographic Dry Condensate Results From Water/Acetone Samples and Aliquots of PAH Samples.

Table 2-3 Gravimetric Dry Condensate Values as a Function of Sample Size and Point of Withdrawal.

Table 2-4 Special Sampling Study.

Table 2-1

Comparison of Puff Numbers from the Standard Four-Port Machine and Six-Port Sequential Smoker

	Number of puffs* to 23 mm butt					
Code	1	2	13	14	15	21
Four-Port Machine	11.0	10.7	9.0	5.8	7.8	6.0
Six-Port Machine	11.6	10.1	8.6	5.8	7.6	5.6

*35 cc, 2 sec., 1/min.

Table 2-2

Chromatographic Dry Condensate Results From Water/Acetone Samples and Aliquots of PAH Samples

Dry Condensate, Wt % Sample

	Aliquot				
Sample Code A	1	2	3	4	Average
H_2O/Acetone Sample	32.3	33.1	36.2	35.3	34.2 %
PAH-1	43.2	41.3	39.0		41.2
PAH-2	35.2	32.8	33.1		33.7
PAH-3	34.3	33.1	35.3		34.2
PAH-4	32.3	32.9	35.9		33.7
Sample Code B					
H_2O/Acetone Sample	34.5	36.4	33.2	35.2	34.8 %
PAH-1	35.7	34.9	37.3		36.0
PAH-2	37.7	37.3	38.3		37.8
PAH-3	40.2	35.5	33.2		36.3
PAH-4	40.8	41.5	51.6		44.6

Table 2-3

Gravimetric Dry Condensate Values as a Function of Sample Size and Point of Withdrawal

Sample No. Order of Withdrawal	Condensate Suspension, gm	Dry Condensate*, gm	Dry Condensate, wt %
1	0.8650	0.264	30.5
2	1.8341	0.568	30.8
3	3.3527	1.050	30.9
4	4.8648	1.524	31.3
5	1.044	0.313	29.9
6	3.028	0.926	30.6
7	6.530	2.081	31.9
8	5.736	1.810	31.6
9	1.014	0.303	29.9
10	1.494	0.446	29.8
11	2.864	0.867	30.3
12	5.183	1.622	31.3
13	1.039	0.306	29.4
14	3.875	1.199	30.9
15	3.874	1.182	30.5
16	6.211	1.949	31.4
17	1.633	0.487	29.7
18	2.777	0.851	30.0
19	5.712	1.783	31.2
20	6.534	2.070	31.7
21	2.311	0.684	29.6
22	3.116	0.942	30.2
23	7.286	2.217	30.4
24	8.876	2.862	32.2
25	5.676	1.753	30.9

*Dried by 48 hour vacuum desiccation.

Table 2-4

Special Sampling Study

Parameter	Individual Bottles 1	2	3	4	5	6
Nicotine (mg/g)	99.9	95.8	100.3	95.5	100.3	97.1
Nicotine Alkaloids (mg/g)	96.2	89.2	87.0	87.0	101.7	95.5
Color Phenols (mg/g)	5.87	----	6.44	6.20	5.94	5.82
GC Phenol (mg/g)	3.72	3.97	4.00	3.63	3.82	3.61
o-Cresol (mg/g)	0.90	1.07	1.02	0.58	0.91	0.90
m-Cresol + p-Cresol (mg/g)	1.65	1.85	1.71	1.31	1.60	1.56
Phenol +Cresols (mg/g)	6.27	6.89	6.73	5.52	6.33	6.07
Weak Acids (meq/g)	1.68	1.53	1.58	1.48	1.65	1.50
Very Weak Acids (meq/g)	0.87	0.84	0.84	0.82	0.97	0.88
Total Acids (meq/g)	2.55	2.37	2.42	2.30	2.62	2.38

Parameter	Avg.	2 x RSD	Δ1-6	Composite	Avg./Comp.
Nicotine (mg/g)	98.1	4.6%	5%	85.4	1.15
Nicotine Alkaloids (mg/g)	92.8	12.8	16	86.4	1.14
Color Phenols (mg/g)	6.05	8.6	10	5.23	1.16
GC Phenol (mg/g)	3.79	8.8	10	3.64	1.04
o-Cresol (mg/g)	0.90	38.1	59,17	0.59	1.52
m-Cresol + p-Cresol (mg/g)	1.61	22.3	34	1.42	1.13
Phenol + Cresols (mg/g)	6.30	15.5	22	5.65	1.12
Weak Acids (meq/g)	1.57	10.4	13	1.36	1.15
Very Weak Acids (meq/g)	0.87	12.3	17	0.86	1.01
Total Acids (meq/g)	2.44	9.9	13	2.22	1.10
					1.15

TABLES

Section 3:00

Table 3-1 Comparative Study Number 1, ORNL Summary (2 pages).

Table 3-2 Comparative Study Number 1, Collaborators Summary.

Table 3-3 Summary of Comparative Study #1.

Table 3-4 Comparative Study #2, Monitor Cigarette.

Table 3-5 Comparative Study #3, NCI Experimental Cigarettes.

Table 3-6 Precision of TPM Determination

Table 3-7 (same page as 3-6) Precision of Nicotine Alkaloid Determination.

Table 3-8 Precision of Water Determination.

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Table 3-10 Confidence Limits (p=0.95) Reported for Selected TRC Analyses Employing Four Smoked Channels.

Table 3-11 (same page as 3-10) Precision of Acetaldehyde and Acrolein Determinations.

Table 3-12 Comparison of Average Results for CO and CO_2 Determined by Dual Column (Original) and Single Column (new) Procedures.

Table 3-13 Precision of CO and CO_2 Determinations, Dual and Single Column Procedures.

Table 3-14 Precision of Formaldehyde Determinations

Table 3-15 (same page as 3-14) Precision of NO_x Determination.

Table 3-16 (same page as 3-14, 3-15) Precision of HCN Determination.

Table 3-17 Percent Dry Condensate Determined Gravimetrically.

Table 3-18 GC Analysis for Residual Water and Acetone in Condensates Dried by Rotary Evaporation.

Table 3-19 (same page as 3-18) Gravimetric versus Chromatographic Dry Condensate Determination.

Section 3:00 Tables (continued)

Table 3-20 Acetone in Spiked Dry Condensate.

Table 3-21 (same page as 3-20) Precision of Condensate Analyses.

Table 3-22 Nicotine Versus Nicotine Alkaloids and Colorimetric Phenols Versus Phenol plus Cresols in Condensates.

Table 3-23 Interlaboratory Comparison of Polycyclic Aromatic Hydrocarbon Data.

Table 3-24 Data Report Formats (2 pages).

Table 3-1

Comparative Study Number 1

ORNL Summary

Particulate Matter Assay	No.of Cigs Analyzed	21-S	22-S	23-S
TPM (mg/cig)	4 x 4	23.36	24.69	35.21
Water (mg/cig)		4.14	4.29	4.30
Nicotine (mg/cig)		1.36	1.41	1.93
Tar (mg/cig)		17.86	18.99	28.98
Colorimetric Phenols (μg/cig)		52.1	58	188
Cigarette Weight (mg)		1138	1018	1102
Pressure Drop (mm H_2O)		122	111	72
No. of Puffs		8.9	8.6	10.8
Butt Length (mm)		28	28	23
Gas Phase Assays				
Oxides of Nitrogen (μg/NO_2/cig)	4 x 1	438	408	240
Cigarette Weight (mg)		1136	1028	1117
Pressure Drop (mm H_2O)		121	112	75
No. of Puffs		9.0	9.0	11.5
Butt Length (mm)		28	28	23
Acetaldehyde (μg/cig)	3 x 1	868	1051	1058
Acrolein (μg/cig)		62	103	104
Isoprene (relative)		0.60	0.96	1.08
Cigarette Weight (mg)		1119	1029	1098
Pressure Drop (mm H_2O)		122	113	72
No. of Puffs		8.3	8.5	10.7
Butt Length (mm)		28	28	23
Hydrogen Cyanide (μg/cig)	4 x 3	31	178	146
Cigarette Weight (mg)		1135	1028	1098
Pressure Drop (mm H_2O)		120	107	68
No. of Puffs		9.3	9.3	10.9
Butt Length (mm)		28	28	23
Formaldehyde (μg/cig)	4 x 3	14.4	30.3	36.8
Cigarette Weight (mg)		1135	1029	1107
Pressure Drop (mm H_2O)		118	111	76
No. of Puffs		8.1	8.0	9.9
Butt Length (mm)		28	28	23
Carbon Monoxide (vol %)	4 x 1	4.0	4.5	3.6
Carbon Dioxide (vol %)	4 x 1	7.9	8.7	7.2
Cigarette Weight		1133	1030	1103
Pressure Drop		109	102	71
No. of Puffs		8.2	8.7	9.7
Butt Length				
Average Experimental Characteristics				
Cigarette Weight Analyzed		1132	1027	1104
Pressure Drop		118	109	72
Number of Puffs		8.6	8.7	10.6

Table 3-2

Comparative Study Number 1

Collaborators Summary

	No.Cigs. Used for Analyses	21 S	22 S	23 S
Cigarette Weight (g)	100	1.133	1.025	1.124
Pressure Drop (cm H_2O)	50	10.8	9.9	6.4
Free Burn Rate (mm/min)	3 x 19	5.2	5.4	4.5
Tar and Nicotine Determination	5 x 20			
No. of Puffs		8.4	8.3	10.8
Butt Length (mm)		28	28	23
Wet Smoke (mg/cig)		22.8	24.8	34.7
Water (mg/cig)		3.3	4.1	3.8
Nicotine (mg/cig)		1.33	1.41	1.97
NFDS (mg/cig)		18.2	19.3	28.9
CO (cc/cig)		16.5	15.9	14.1
Total Phenols (μg/cig)		62	73	275
pH of Particulate Smoke	3 x 3	5.8	5.8	5.7
HCN of Total Aldehydes	5 x 20			
No. of Puffs		8.0	8.0	10.1
HCN-Total Smoke (μg/cig)		159	314	320
HCN-Gas Phase (μg/cig)		88	187	180
Aldehydes-Gas Phase (μg/cig)		1090	1590	1630
Aldehydes by G.C.	3 x 6			
Acetaldehyde (μg/puff)		88	140	110
Acrolein (μg/puff)		5.0	11	9.0
Acetone (μg/puff)		24	54	45
Simultaneous Determination (NDIR)	3 x 12			
NO (cc/puff)		0.045	0.045	0.029
CO_2 (cc/puff)		3.8	3.8	3.2
CO (cc/puff		2.1	2.0	1.4
Chemical Analysis of Tobacco (Wet Basis)				
Nicotine (%)	40	1.76	1.76	1.64
Nitrate (%)	40	0.29	0.29	0.20
Moisture (%)	3 x 20	10.8	10.7	10.8

Table 3-3

Summary of Comparative Study #1

	21-S		22-S		23-S	
Particulates	ORNL	Coll.	ORNL	Coll.	ORNL	Coll.
TPM mg/cig	23.4	22.8	24.7	24.8	35.2	34.7
Water mg/cig	4.1	3.3	4.3	4.1	4.3	3.8
Nicotine mg/cig	1.36	1.33	1.41	1.41	1.93	1.97
Tar mg/cig	17.9	18.2	19.0	19.3	29.0	28.9
Color Phenols μg/cig	62*	62	70*	73	226*	275
Gas Phase						
Oxides of Nitrogen (μg NO_2/cig)**	438	755	408	751	240	618
Hydrogen Cyanide (μg/cig)	31	88	178	187	146	180
Formaldehyde (μg/cig)	14.4		30.3		36.8	
Acetaldehyde (μg/cig)**	868	704	1051	1120	1058	1111
Acrolein (μg/cig)**	62	40	103	88	104	91
Isoprene (relative)	0.60		0.96		1.08	
Acetone (μg/cig)**		192		432		454
Carbon Monoxide (vol %)**	4.0	5.5	4.5	5.2	3.6	3.7
Carbon Dioxide (vol %)**	7.9	9.9	8.7	9.9	7.2	8.4
Experimental Characteristics						
Average Cigarette Weight (mg)	1132	1133	1027	1025	1104	1124
Average Pressure Drop (mm H_2O)	118	108	109	99	72	64
Average Number of Puffs	8.6		8.7		10.6	
Particulate Assay		8.4		8.3		10.8
Gas Phase Assays		8.0		8.0		10.1

*Multiplied by collaborator's factor of 1.2 to compare results on same basis.
**Reported values computed from collaborator's submission to express results in same units.

Table 3-4

Comparative Study #2

Monitor Cigarette

Per Cigarette Deliveries

Parameter	Collaborator	ORNL
Total Particulate Matter	24.9 mg	26.0 mg
Nicotine	1.43 mg	1.39mg
Water	4.5 mg	4.6 mg
Tar	19.0 mg	20.0 mg
Phenol	36.0 mg	----
Carbon Monoxide	14.3 mg	14.1 mg
Acetaldehyde	1005 μg	1009 μg
Acrolein	111 μg	108 μg
Formaldehyde	40.8 μg	40.1 μg
Hydrogen Cyanide	269.2 μg	202 μg
Oxides of Nitrogen	260.3 μg	236 μg
Acids		
Strong	0.005 meq	----
Weak	0.027 meq	----
Very Weak	0.022 meq	----
Puffs per Cigarette	9.5	9.3

Table 3-5

Comparative Study #3

NCI Experimental Cigarettes

Code No.	Nicotine (mg/cig)		FTC Tar (mg/cig)	
	ORNL	Collab.	ORNL	Collab.
2	1.68	1.91	25.9	26.7
5	1.65	1.69	24.2	23.4
8	2.48	2.31	34.6	32.1
9	1.42	1.42	20.8	19.7
13	0.29	0.38	13.2	11.5
14	0.20	0.22	11.3	10.0
15	0.76	0.66	15.4	15.0
16	0.89	0.88	15.5	14.8
17	0.70	0.74	11.7	11.1
18	0.78	0.80	13.3	12.1
19	0.84	0.92	15.1	14.1
20	1.98	1.72	30.0	29.1
21	0.16	0.26	5.2	5.3

Table 3-6

Precision of TPM Determination

Analysis	Cigs. per port		Avg. No. Ports		Avg. Coefficient of Variation (%)		Avg. Relative Standard Error		Avg. Confidence Interval-95 (%)	
	1	2	1	2	1	2	1	2	1	2
Units										
mg/cig.	4	4	4.7	5.1	3.1	2.7	1.4	1.2	4.5	3.6
mg/puff	4	4	4.7	5.1	3.5	2.9	1.6	1.3	5.0	3.8
mg/g tob.	4	4	4.7	5.1	3.1	2.8	1.5	1.2	4.5	3.7

Table 3-7

Precision of Nicotine Alkaloids Determination

Analysis	Cigs. per port		Avg. No. Ports		Avg. Coefficient of Variation (%)		Avg. Relative Standard Error		Avg. Confidence Interval-95 (%)	
Units										
mg/cig.	4	4	5.0	4.6	3.6	3.1	1.6	1.4	5.0	4.5
mg/puff	4	4	5.0	4.6	4.0	3.7	1.8	1.7	5.3	5.4
mg/g tob.	4	4	5.0	4.6	3.7	3.1	1.6	1.5	5.0	4.5

Table 3-8

Precision of Water Determination

Analysis	Cigs. per Port		Avg. No. Ports		Avg. Coefficient of Variation (%)		Avg. Relative Standard Error		Avg. Confidence Interval-95 (%)	
	1	2	1	2	1	2	1	2	1	2
Units										
mg/cig.	4	4	5.0	5.0	8.3	10.6	3.7	4.7	11.3	13.9
mg/puff	4	4	5.0	5.0	8.0	10.6	3.6	4.7	10.9	14.0
mg/g tob.	4	4	5.0	5.0	8.2	10.6	3.7	4.7	11.2	13.8

Table 3-9

Precision of Tar Determination

Analysis	Cigs. per Port		Avg. No. Ports		Avg. Coefficient of Variation (%)		Avg. Relative Standard Error		Avg. Confidence Interval-95 (%)	
	1	2	1	2	1	2	1	2	1	2
Units										
mg/cig.	4	4	4.6	5.2	3.1	3.1	1.4	1.4	4.6	4.2
mg/puff	4	4	4.6	5.2	3.5	3.3	1.6	1.5	5.2	4.2
mg/g tob.	4	4	4.6	5.2	3.1	3.2	1.4	1.4	4.6	4.2

Table 3-10

Confidence Limits (p = 0.95) Reported for Selected TRC Analyses
Employing Four Smoked Channels

Analysis	Average Delivery	Cigs. Per Channel (Port)	CI	% of Avg.
TPM	35 mg/cig.	5	± 2.0 mg	5.7
Tar	32 mg/cig.	5	± 1.8 mg	5.6
Nicotine Alkaloids	1.5 mg/cig.	5	± 0.12 mg	8.0
Whole Smoke HCN	300 µg/cig.	5	± 15 µg	5.0
Steam Volatile Phenols in TPM	240 µg/cig.	5	± 2 µg	0.8
Phenol, cold trapped	111 µg/cig.	20	± 3.0 mg	2.7
o-Cresol, cold	15 µg/cig.	20	± 2.2 mg	14.7
Total Volatile Aliphatic Aldehydes methanol trapped	2.4 mg/cig.	5	± 0.10 mg	4.2
Total Steam Volatile Acids cold trapped	1.6 mg/cig.	10	± 0.10 mg	6.2

From Reference No. 7.

Table 3-11

Precision of Acetaldehyde and Acrolein Determinations

	Cigs. per Port		Avg. No. Ports		Avg.Coeff. Var.(%)		Avg.Rel.Std. Error(%)		Avg.CI_{95}	
	Acet.	Acrol.	Acet.	Acrol.	Acet.	Acrol.	Acet.	Acrol.	Acet.	Acrol.
µg/cig.	1	1	3	3	2.6	2.8	1.5	1.6	6.5	6.9
µg/puff	1	1	3	3	3.9	4.6	2.3	2.6	9.8	11.4
µg/g tob.	1	1	3	3	2.6	2.8	1.5	1.6	6.5	7.0

Table 3-12

Comparison of Average Results for CO and CO_2 Determined by Dual Column (Original) and Single Column (New) Procedures

milliliters per cigarette

Sample Code	Carbon Monoxide		Carbon Dioxide	
	Dual	Single	Dual	Single
1	18.6	16.8	34.0	31.8
2	16.2	16.1	29.2	32.5
3	13.7	13.4	27.7	27.2
4	19.3	20.1	33.5	37.5
5	15.2	14.0	29.8	30.0
15	19.5	19.4	23.0	24.4
16	15.5	13.8	25.3	25.4
17	11.7	11.8	19.9	21.6
18	15.0	13.9	21.6	22.2
19	18.0	13.5	23.1	20.2
20	25.7	20.1	40.8	35.1
21	9.6	11.3	13.6	15.1
22	25.8	22.4	27.3	30.1
23	22.4	21.4	25.1	27.1

Table 3-13

Precision of CO and CO_2 Determinations

Dual and Single Column Procedures

Carbon Monoxide

Procedure	Cigs. per Port		Avg. No. Ports		Avg. Coeff. Var. %		Avg.Rel.Std. Error %		Avg. Confidence Int.-95 %	
	Dual	Single	Dual	Single	Dual	Single	Dual	Single	Dual	Single
ml/cig.	1	1	4.4	6.5	6.0	7.9	2.9	3.1	9.3	8.3
ml/puff	1	1	4.4	6.5	4.5	6.3	2.1	2.5	6.7	6.5
ml/g tob.	1	1	4.4	6.5	6.2	7.9	2.9	3.1	9.7	8.3

Carbon Dioxide

Procedure	Cigs. per Port		Avg. No. Ports		Avg. Coeff. Var. %		Avg.Rel.Std. Error		Avg. Confidence Int.-95 %	
	Dual	Single	Dual	Single	Dual	Single	Dual	Single	Dual	Single
ml/cig.	1	1	4.1	6.1	5.1	6.1	2.5	2.5	8.7	6.6
ml/puff	1	1	4.1	6.1	2.8	4.6	1.4	1.8	4.6	4.9
ml/g tob.	1	1	4.1	6.1	5.2	6.1	2.6	2.5	8.9	6.6

Table 3-14

Precision of Formaldehyde Determinations

Analysis	Cigs. per Port 1	Cigs. per Port 2	Avg. No. Ports 1	Avg. No. Ports 2	Avg. Coeff. Var. % 1	Avg. Coeff. Var. % 2	Avg.Rel.Std. Error % 1	Avg.Rel.Std. Error % 2	Avg. Confidence Int.-95 % 1	Avg. Confidence Int.-95 % 2
µg/cig.	3	3	3.8	4.3	2.9	4.9	1.5	2.4	5.1	7.6
µg/puff	3	3	3.8	4.3	3.7	4.7	1.9	2.3	6.4	7.4
µg/g tob.	3	3	3.8	4.3	2.9	4.9	1.5	2.4	5.0	7.7

Table 3-15

Precision of NO_x Determination

Analysis	Cigs. per Port 1	Cigs. per Port 2	Avg. No. Ports 1	Avg. No. Ports 2	Avg. Coeff. Var. % 1	Avg. Coeff. Var. % 2	Avg.Rel.Std. Error % 1	Avg.Rel.Std. Error % 2	Avg. Confidence Int.-95 % 1	Avg. Confidence Int.-95 % 2
µg/cig.	1	1	5.0	4.7	5.2	3.9	2.3	1.8	6.8	5.4
µg/puff	1	1	5.0	4.7	5.2	4.1	2.4	1.9	6.7	5.6
µg/g tob.	1	1	5.0	4.7	5.5	4.1	2.5	1.9	7.3	5.6

Table 3-16

Precision of HCN Determination

Analysis	Cigs. per Port 1	Cigs. per Port 2	Avg. No. Ports 1	Avg. No. Ports 2	Avg. Coeff. Var. % 1	Avg. Coeff. Var. % 2	Avg.Rel.Std. Error % 1	Avg.Rel.Std. Error % 2	Avg. Confidence Int.-95 % 1	Avg. Confidence Int.-95 % 2
µg/cig.	3	3	4.8	4.4	3.7	5.1	1.7	2.4	5.3	7.8
µg/puff	3	3	4.8	4.4	4.4	5.2	2.0	2.5	6.2	7.9
µg/g tob.	3	3	4.8	4.4	4.0	5.1	1.8	2.4	5.6	7.8

Table 3-17

Percent Dry Condensate Determined Gravimetrically

Code No.	Wet wt. g.	Dry wt. g.	Drying time hr.	Dry condensate %	
			Rotary Evaporator		
10 F-A	13.239	4.045	16	30.55	
10 F-B	12.916	3.838	2	29.56	
10 F-C	12.916	4.239	3.25	32.66	
10 F-D	14.717	4.673	6	31.75	
					31.1
1 F 1	17.140	4.249	2.5	24.79	
1 F 2	21.468	5.072	16	23.63	
1 F 4	14.898	3.646	3	24.47	
13 F 2	12.535	1.188	2	9.45	
13 F 3	7.844	.758	2	9.66	
			Vacuum Desiccator Drying*		
10 F-1	0.290	0.084	7 days	28.6	
10 F-2	0.486	0.132	11 days	27.2	
10 F-3	0.372	0.111	18 days	29.8	
10 F-4	0.703	0.224	25 days	31.9	
					29.4
10 F-11	2.580	0.793		30.7	
10 F-12	1.067	0.316		29.2	
10 F-13	1.524	0.466		30.6	
10 F-14	2.072	0.602		29.0	
					29.9

*To constant weight, 11-18 days.

Table 3-18

GC Analysis for Residual Water and Acetone in Condensates Dried by Rotary Evaporation

Sample*	Weight (mg)	Water (mg)	Acetone (mg)	Water (%)	Acetone (%)	Water + Acetone %
10 FA-1	41	0	0	0	0	0
10 FA-2	147	0.19	0.19	0.13	0	0.1
10 FB-1	133	1.56	1.08	1.17	0.81	2.0
10 FB-2	98	0.78	0.43	0.80	0.44	1.2
10 FC-1	129	1.75	0.87	1.37	0.67	2.0
10 FC-2	134	0.55	0.35	0.41	0.26	0.7
10 FD-1	121	1.05	0.40	0.87	0.33	1.2
10 FD-2	96	0.39	0.38	0.41	0.40	0.8

*Rotary evaporation of 120-140 mm, 37°C, for 16, 2, 3.25 and 6 hours, A, B, C, D, respectively.

Table 3-19

Gravimetric versus Chromatographic Dry Condensate Determination

Percent Dry Condensate

Sample	Gravimetric* wt % sample	Chromatographic* wt % sample	
		Run 1	Run 2
9F	25.9	19.9	22.9
7F	38.6	30.5	33.3
6F	32.6	27.8	28.4
14F	21.4	14.4	19.2
2F	29.6	25.3	26.3

*Each result is the average from 4 determinations (aliquots).

Table 3-20

Acetone in Spiked Dry Condensate

Sample No.	Weight Dry Condensate	Acetone Added	Acetone Found
	(gm)	(mg)	(mg)
1	0.402	119	122
2	0.530	143	147
3	0.337	79	80
4	0.480	151	151
5	1.591	781	805
6	2.218	1006	1018
7	2.473	1132	1139

Table 3-21

Precision of Condensate Analyses[1]

	Avg. Coeff. Variation[2]	Avg. Relative Std. Error[3]
Water[4]	5.0	2.6
Acetone[4]	3.8	1.9
Dry Condensate[4]	3.8	1.9
Nicotine Alkaloids	4.1	2.1
Nicotine	3.9	2.0
Colorimetric Phenols	4.6	2.4
Phenol	8.0	4.2
o-Cresol	14.4	7.6
m-Cresol + p-Cresol	10.7	5.7
Phenol + Cresols	6.0	2.2
Weak Acids	7.9	4.1
Very Weak Acids	8.0	4.2
Weak plus Very Weak Acids	5.8	2.1
Phenanthrene	9.7	5.1
Benz(a)anthracene	10.1	5.3
Benzo(a)pyrene	13.3	7.2
pH	0.9	0.6

[1]Condensate suspensions aliquoted from large scale batch prepared for bioassay.
[2]Percent of mean.
[3]Approximately 4 determinations per result.
[4]Chromatographic only.

Table 3-22

Nicotine Versus Nicotine Alkaloids and Colorimetric Phenols Versus Phenol plus Cresols in Condensates

Milligrams per Gram of Dry Condensate

Sample Code	Nicotine	Nicotine Alkaloids	Colorimetric Phenols	Phenol + Cresols
1	97.1	94.3	6.86	7.59
2	95.4	93.6	6.55	6.96
3	99.2	94.6	6.90	7.12
4	88.4	84.5	5.99	6.38
5	92.1	92.8	5.81	6.10
6	91.8	88.6	6.37	6.52
7	98.4	93.1	6.10	6.72
8	93.3	90.0	6.18	6.37
9	89.7	82.4	5.75	6.20
10	112.7	108.3	7.34	8.71
11	111.9	114.3	7.45	7.34
12	153.5	157.0	5.35	6.00
13	42.1	39.8	11.74	12.44
14	31.6	30.3	8.67	9.39
15	60.0	56.6	5.19	5.44

Table 3-23

Interlaboratory Comparison of Polycyclic Aromatic Hydrocarbon Data

Parts Per Million, Dry Condensate

Sample Code	Phenanthrene		Benz(a)anthracene		Benzo(a)pyrene	
	ORNL	Collab.	ORNL	Collab.	ORNL	Collab.
1	25.7	22.4	1.3	1.3	0.8	0.7
2	23.2	21.6	1.2	1.4	0.7	0.7
3	18.0	19.0	1.1	1.2	0.8	0.8
4	20.1	26.3	1.4	1.4	0.9	0.8
5	16.6	21.2	1.0	1.0	0.9	0.8
6	18.8	24.8	1.4	1.5	0.9	1.0
7	17.6	18.6	1.3	1.0	0.9	0.7
8	21.3	25.3	1.3	1.4	0.8	0.8
9	17.1	19.0	1.0	1.0	0.7	0.6
10	---	25.5	1.1	1.2	0.8	0.8
13	22.9	24.0	1.0	1.5	1.0	1.0
14	17.5	16.4	1.2	1.1	0.8	0.6
15	---	15.8	1.0	1.4	1.0	1.0
16	18.7	18.6	1.0	0.9	0.5	0.5
17	18.0	11.2	0.8	0.7	0.6	0.4

Table 3-24

Data Report Formats

Parameter Determined, units

I	Code	Port 1	Port 2	Port 3	Port 4	Port 5	Port 6	Number	Avg.	Std. Error	CI_{95} %

Parameter, Determined, units

		Run I			Run II			Combined			
II	Code	Number	Avg.	Std.Error	Number	Avg.	Std.Error	Number	Avg.	Std.Error	CI_{95} %

Parameter Determined, mean ± 95% CI

III[1]	Code	quant./cig.	CI %	Quant./puff	CI %	Quant./1 smoke	CI %	Quant./g tob.	CI %	wt % TPM	

Parameter Determined, number determinations, avg.coefficient variations, avg.relative standard deviation

		quant./cig.			quant./puff			quant./1 smoke			quant./g.tob.burned		
IV[1]	Code	N	CV	RSE	N	CV	RSE	N	CV	RSE	N	CV	RSE

Parameter Determined, units

average, avg. std. error, avg. 95 % CI, avg. coeff. var., avg. rel. std. error

V[2]	Code	No.Lots	No. determinations	Average	Std. Error	95% CI %	CV	R. S. E.

Experimental Characteristics[3]

VI	Code	TPM	Water	Nicotine	Tar	Acetaldehyde	Acrolein	Formaldehyde	HCN	NO_x	CO	CO_2

[1]Smoke, gas phase, TPM analyses only.
[2]Condensate analyses only.
[3]Cigarette weight, no. puffs, RTD, for analyzed cigarettes.

Table 3-24

Data Report Formats
(continued)

Smoke Parameters

Average per Cigarette[4]

VII	Code	TPM	Water	Nicotine	Tar	Acetaldehyde	etc. . . . .
		mg	mg	mg	mg	µg	

Condensate Parameters

Average Analyses

VIII	Code	Water	Acetone	Dry Cond.	Phenol	etc. . . . .
		%	%	%	mg/gm	

[4]also /puff, /l smoke, /g tob. burned, 95% CI, Coeff. Var., RSE, N

TABLES

Section 4:00

Table 4-1 Peak Identifications for TPM and Gas Phase Profiles.

Table 4-2 Reproducibility of Relative Peak Heights in TPM Profile.

Table 4-3 Quantitative Estimation of Sulfur Components in Smokes.

Table 4-4 Quantitative Estimation of Trace Metals In Smoke Condensates.

Table 4-1

Peak Identifications for TPM and Gas Phase Profiles

TPM Profile
(Fig. 4-4 and 5-3)

Peak No.	Identification
3	Phenol
6,7	Cresols
13	Glycerol
14	Catechol + (?)
15	Nicotine
17	Hydroquinone + (?)
40	Palmitic Acid
42	Oleic, Linoleic, Linolenic acid
43	Stearic acid

Gas Phase Profile
(Fig. 5-4)

Peak No.	Identification
1	Isoprene
2	Acetaldehyde
3	Furan
4	Formaldehyde + Methyl formate
5	Propionaldehyde + 2 methyl-furan
6	Isobutyraldehyde
7	Ethyl formate
8	Methyl acetate
9	Acrolein
10	Acetone
11	Methanol
12	Ethyl acetate
13	Benzene
14	Ethanol
15	Acrylonitrile

Table 4-2

Reproducibility of Relative Peak Heights in TPM Profile

Peak No.	Peak Ht. Ratio Peak x / Peak 17	Coeff. of* Variation
1	1.29	9
2	0.11	16
3	0.32	6
4	0.22	2
5	0.20	3
6	0.35	1
7	0.23	4
9	0.17	13
12	0.17	12
13	9.91	4
15	3.12	2
18	0.41	2
19	0.19	4
21	0.61	3
24	0.25	6
26	0.24	8
28	0.16	14
29	1.04	3
31	1.23	3
33	0.70	6
40	0.72	4
41	0.67	7
42	0.87	2
43	0.53	8

*From 8 determinations (ports), 4 cigarettes per port.

Table 4-3

Quantitative Estimation of Sulfur Components in Smokes

Cigarette	Micrograms per Cigarette			
	COS	H_2S	CS_2	SO_2
Kentucky Reference-1[a]	30	38	3.8	< 1
Kentucky Reference-2	32	90	4.7	< 1
Brand A-Charcoal Filter	18	27	1.9	3.7
Brand B-Little Cigar Cellulose Filter	21	109[b]	3.6	3.9
Brand C-85 mm Commercial	23	79	4.5	3.7
Marijuana	23	80	2.0	< 1

[a]Smoked on "Hexapuffer".

[b]Estimate-on Curved Portion of Calibration Graph.

Table 4-4

Quantitative Estimation of Trace Metals in Smoke Condensates

Sample No.	Cd	Cr	Cu	Ni	Pb	Zn	Hg*
			PPM				
1	10.0	0.4	3.2	0.71	6.9	14	55
2	4.2	0.2	2.0	0.71	3.1	12	45
3	1.7	< .2	3.6	0.28	7.4	44	68
4	6.2	0.3	6.2	1.0	8.9	25	45
5	6.2	0.3	3.8	0.33	4.9	50	49
6	10.8	0.4	5.6	1.2	4.0	28	51
7	9.2	≤ .2	5.0	2.6	3.6	25	62
8	7.0	2	5.6	1.2	3.6	22	186
9	8.0	0.6	5.3	0.88	7.1	32	86
10				2.8	2.9		49
13	5.1	0.4	7.5	0.6	1.3	31	195
14	4.2	0.9	6.1	1.0	2.4	30	46
15	7.5	0.9	6.1	2.3	6.5	47	55
16	8.3	0.8	6.0	0.83	4.8		445
18	6.1	0.7	6.8	0.69	5.3	40	445
19	5.2	0.8	21	1.21	13	49	77
20		0.7	5.5	1.6		35	74
22	4.9	0.6	3.8	0.91	9.5	21	59
23	10.0	0.8	5.2	2.7	12	30	34

*parts per billion

TABLES

Section 5:00

Table 5-1 Chemical Characteristics of Smokes Delivered by Ammonium Sulfamate Treated Cigarettes.

Table 5-2 Chemical Characteristics of Smoke Condensates and Condensate Extracts from Ammonium Sulfamate Treated Cigarettes.

Table 5-3 Preliminary Survey of Chemical Composition of Marijuana and Marijuana Smoke. (3 pages).

Table 5-4 Simulated Dog Puffing of a Kentucky Reference Cigarette.

Table 5-5 Chemical Characterization of ADL Machine.

Table 5-6 Relative Peak Heights in TPM Profiles from ADL Machine, Simulated Animal Smoking, and Standard Analytical Smoking.

Table 5-7 Relative Distribution of Smoke Components: ADL, Analytical, and Simulated Animal Smoking.

Table 5-8 (same page as 5-7) Comparison of Dose Offered: ADL Machine versus Simulated Animal Smoking.

Table 5-1

Chemical Characteristics of Smokes Delivered by Ammonium Sulfamate Treated Cigarettes

Average Results

Parameter[1]		Control AS-6-A	Concentration Low AS-6-C	Concentration High AS-6-C	Control 8A-OPM	Concentration Low 8B-OPM	Concentration High 8C-OPM	Control[2] 8A-OPM	High[2] 8C-OPM
Particulate Matter									
TPM	mg/cig	24.8	32.0	31.5	24.7	27.4	33.8	24.6	33.5
Water	mg/cig				3.8	3.9	5.6	2.9	4.2
Nicotine Alkaloids	mg/cig	0.86	1.19	1.17	0.64	1.16	1.35	0.88	1.20
Tar	mg/cig				20.3	22.4	26.8	20.8	28.1
Gas Phase									
Acetaldehyde	mg/cig	---	---	---	1.14	1.03	1.04	---	---
Acrolein	mg/cig	---	---	---	0.12	0.10	0.10	---	---
Isoprene	relative	---	---	---	0.44	0.37	0.45	---	---
Formaldehyde	μg/cig	33	32	28	27	19	24	---	---
Oxides of Nitrogen	mg/cig	0.31	0.38	0.35	0.45	0.48	0.50	---	---
Hydrogen Cyanide	mg/cig	0.18	0.23	0.23	0.17	0.14	0.15	---	---
Carbon Monoxide	vol %	5.0	8.0	6.5	---	---	---	5.0	8.2
Carbon Dioxide	vol %	9.0	9.6	9.0	---	---	---	9.7	9.7

[1]AS and OPM series prepared similarly but at different times.

[2]Hand selected by submitter for independent study.

Table 5-2

Chemical Characteristics of Smoke Condensates and Condensate Extracts from Ammonium Sulfamate Treated Cigarettes

Average Results

Parameter		Condensate Extracts			Condensate Extracts			Whole Condensate		
		AS-6-A	AS-6-B	AS-6-C	8A-OPM	8B-OPM	8C-OPM	8A-OPM	8B-OPM	8C-OPM
Nicotine	mg/gm	35	65	46	36	35	37	49	---	61
Nicotine Alkaloids	mg/gm				21	30	27	44	49	59
Colorimetric Phenols	mg/gm	8.6	10.8	10.5	9.1	8.3	9.8	5.5	5.3	6.4
Phenol	mg/gm	4.6	6.1	5.7	5.4	6.0	6.1	2.9	2.9	4.6
o-Cresol	mg/gm	1.2	1.4	1.7	2.9	2.5	3.1	0.9	0.9	1.3
m-Cresol + p-Cresol	mg/gm	2.8	2.9	4.7	2.9	2.9	3.4	1.4	1.4	2.1
Phenol + Cresols	mg/gm	8.6	10.4	12.0	11.3	11.5	12.5	5.2	5.1	8.0
Weak Acids	meq/gm	0.67	0.65	0.69	0.63	0.61	0.61	1.29	1.35	1.42
Very Weak Acids	meq/gm	0.89	0.86	0.98	1.00	0.85	0.80	0.94	0.95	1.08
Total Weak Acids	meq/gm	1.56	1.51	1.67	1.63	1.46	1.41	2.23	2.30	2.50
Phenanthrene	µg/gm	55	79	69				22	17	21
Benz(a)anthracene	µg/gm	3.3	3.1	3.3				1.8	1.6	1.7
Benzo(a)pyrene	µg/gm	2.5	2.3	2.5				1.3	1.2	1.2

Table 5-3

Preliminary Survey of Chemical Composition of Marijuana and Marijuana Smoke

	Average Delivery per.cig.	Average Delivery std.dev.	No.of Ports	No.of Cigs.	Avg.Cig. wt.	Avg.RTD (in H_2O)	Avg.No. Puffs
Particulate Matter							
TPM	26.5 mg	2.10	15	60	1098 mg	4.6	10.8
Water	2.0	0.84	12	48	1098 mg	4.7	10.8
Cannabidiol	0.33 mg	0.03	7	28	1099 mg	4.5	10.7
Δ^9-THC	0.73 mg	0.03	7	28	1099 mg	4.5	10.7
Cannabinol	0.36 mg	0.07	7	28	1099 mg	4.5	10.7
Gas Phase							
Acetaldehyde	1.13 mg	---	2	2	1137 mg	4.9	11.0
Acrolein	0.14 mg	---	2	2	1137 mg	4.9	11.0
Carbon Monoxide	3.2 vol%	0.12	3	3	1099 mg	5.2	10.0
Carbon Dioxide	6.3 vol%	0.16	3	3	1099 mg	5.2	10.0
Oxides of Nitrogen	77 µg	5.6	5	5	1091 mg	5.1	10.0
Hydrogen Cyanide	219 µg	16.5	4	8	1096 mg	5.0	10.5
Formaldehyde	28.6 µg	1.9	3	9	1096 mg	5.1	10.9
pH, Avg.	5.53		4	4	1070 mg	---	10.7
puff 1	----						
2	5.72	0.06	4	4	1070 mg	---	10.7
3	5.71	0.07	4	4	1070 mg	---	10.7
4	5.68	0.05	4	4	1070 mg		10.7
5	5.54	0.11	4	4	1070 mg		10.7
6	5.54	0.10	4	4	1070 mg		10.7
7	5.51	0.13	4	4	1070 mg		10.7
8	5.47	0.16	4	4	1070 mg		10.7
9	5.47	0.16	4	4	1070 mg		10.7
10	5.38	0.14	4	4	1070 mg		10.7
11	5.24	0.11	4	4	1070 mg		10.7

Table 5-3 (Continued)

Result, per gm. dry cond.

	Lot 1	Lot 3	Average
Cannabidiol	----	18.1 ± 0.3 mg	18.1 mg
Δ^9-THC	----	39.6 ± 0.3 mg	39.6 mg
Cannabinol	----	17.3 ± 0.4 mg	17.3 mg
Phenol	3.51 mg	3.45 ± 0.11 mg	3.48 mg
o-Cresol	0.70 mg	0.70 ± 0.02 mg	0.70 mg
m + p Cresol	3.30 mg	2.98 ± 0.20 mg	3.14 mg
Phenol + Cresols	7.51 mg	7.13 ± 0.26 mg	7.32 mg
Color. Phenol	6.00 mg	5.62 ± 0.10 mg	5.81 mg
Weak Acids	1.26 meq	1.23 ± 0.12 meq	1.24 meq
Very Weak Acids	1.26 meq	1.54 ± 0.16 meq	1.38 meq
Total Acids	2.52 meq	2.77 ± 0.28 meq	2.62 meq
Phenanthrene	36.3 μg	46.9 ± 0.13 μg	41.6 μg
BaA	3.96 μg	3.14 ± 0.11 μg	3.55 μg
BaP	2.60 μg	2.10 ± 0.06 μg	2.35 μg
pH	4.60	4.63	4.62

Table 5-3 (Continued)

Inorganic Constituents of Marijuana and Marijuana Smoke Condensate

Element	Filler (ppm)	Techniques	Condensate (ppm)	Technique
Sodium	440	NAA	63	NAA
Potassium	31,000	NAA		
Rubidium	8.6	NAA		
Cesium	0.3	NAA		
Barium	140	NAA		
Lanthanum	2.3	NAA		
Cerium	2.5	NAA		
Samarium	0.32	NAA		
Europium	0.058	NAA		
Scandium	0.3	NAA		
Hafnium	0.036	NAA		
Iron	640	NAA		
Antimony	0.34	NAA		
Aluminum			16.7	NAA
Chlorine			424	NAA
Manganese			1.1	NAA
Calcium				
Mercury			0.063	AA
Zinc			150*	MS
Copper			2600*	MS
Nickel			110*	MS
Lead			36	MS
Cadmium			4	MS
Bromine	20	NAA		
Chromium	4.4	NAA	0.14	NAA
Cobalt			0.02	NAA

NAA - Neutron Activation Analysis
MS - Spark Source Mass Spectrometry
AA - Atomic Absorption

*possible contamination

Table 5-4

Simulated Dog Puffing of a Kentucky Reference Cigarette

Respirator Setting	Minute Volume[3]	TPM mg	Nicotine mg	Water mg	Tar mg	CO ml	CO_2 ml	Acetaldehyde mg	Acrolein mg	Time[4] min.
					Per Cigarette					
20 cc/50 per min.	1000 cc	118	4.2	34.8	81.3	34.8	84.0	2.4	0.34	1.8
9 / 111	1000	127	5.4	38.6	83.0	31.1	97.0	---	---	2.0
5 / 200	1000	124	5.2	39.1	80.1	31.3	105.6	2.8	0.28	1.9
2 / 60	120	28	0.9	3.9	23.2	---	---	---	---	6.4
5 / 60	300	45	2.0	12.9	30.0	---	---	---	---	3.6
5 / 90	450	48	2.0	14.4	31.6	---	---	---	---	3.0
10 / 60	600	62	2.5	24.3	35.2	---	---	---	---	2.5
Continuous[1]	1000	126	5.5	62.9	57.6	---	---	---	---	1
35/1[2]	35	45	2.9	6.6	35.5	---	---	---	---	10

---

[1] 1 liter water bottle drawing continuous puff for 1 min.
[2] Analytical smoking.
[3] Nominal from settings.
[4] Time to 23 mm butt.

Table 5-5

Chemical Characterization of ADL Machine

Per Cigarette

System	TPM	Nicotine	Water	Tar	CO	CO_2	Acetaldehyde	Acrolein	Phenolics
	mg	mg	mg	mg	ml	ml	mg	mg	mg
ADL Machine									
Into syringe	61.3	3.5	9.6	48.2	---	---	---	----	0.29
Syringe exhaust	49.0	3.1	6.3	39.6	20.9	46.6	1.4	0.17	0.27
Holding tube exhaust	45.8	2.9	4.6	38.3	20.5[3]	42.2[3]	1.1	0.16	---
Analytical Machine	65.6	3.7	10.1	51.8	18.5	41.4	1.4	0.13	0.32
Analytical Machine[2]	43.2	2.5	5.5	35.2	---	---	---	---	---

[1]Two puffs per minute, 35 cc volume, 2 sec. duration, Kentucky Ref. Cig. (1R1), 4-8 cigarettes per determination.
[2]One puff per minute.
[3]Blown into sampling bag on subsequent puff, all others respirated from tube.

Table 5-6

Relative Peak Heights in TPM Profiles from ADL Machine, Simulated Animal Smoking, and Standard Analytical Smoking

Peak No.	Peak Ht. Ratio (Peak x : Peak 17) ADL Machine Into Front Valve	Out of Front Valve	Out of Holding Tube	Simulated Animal Smoke	Analytical Standard Smoke
1	0.23	0.25	0.23	0.27	0.22
2	0.12	0.09	0.06	0.08	0.13
3	0.38	0.34	0.32	0.38	0.37
4	0.22	0.23	0.22	0.24	0.23
5	0.18	0.18	0.18	0.20	0.19
6	0.39	0.37	0.37	0.38	0.38
7	0.26	0.24	0.23	0.24	0.25
9	0.20	0.18	0.17	0.21	0.18
12	0.19	0.19	0.18	0.15	0.13
13	10.20	10.47	9.94	7.22	9.40
15	3.32	3.20	2.99	2.65	3.00
18	0.37	0.35	0.38	0.35	0.39
19	0.21	0.21	0.22	0.22	0.21
21	0.54	0.54	0.52	0.61	0.59
24	0.21	0.22	0.23	0.22	0.24
26	0.17	0.19	0.19	0.19	0.21
28	0.15	0.17	0.17	0.16	0.15
29	0.98	0.91	0.97	0.95	1.03
31	1.09	1.11	1.09	1.25	1.17
33	0.62	0.63	0.65	0.62	0.66
40	0.73	0.69	0.78	0.69	0.78
41	0.50	0.50	0.49	0.67	0.60
42	0.89	0.83	0.88	0.87	0.90
43	0.26	0.23	0.27	0.24	0.29

Table 5-7

Relative Distribution of Smoke Components

ADL, Analytical, and Simulated Animal Smoking

Component Ratios

x : Tar

System	TPM	Nicotine	Water	CO	CO_2	Acetal-dehyde	Acro-lein	CO(ml) / CO_2(ml)	Acetal-dehyde(mg) / Acrolein (mg)	Nicotine(mg) / CO(ml)
	mg/mg			ml/ml		mg/mg				
Analytical	1.27	0.071	0.194	0.36	0.80	0.027	0.0025	0.45	10.8	0.20
ADL[1]	1.20	0.076	0.120	0.53	1.01	0.028	0.0042	0.48	6.9	0.14
Dog Puffing[2]	1.51	0.060	0.460	0.40	1.17	0.032	0.0038	0.34	8.4	0.15

[1]Holding tube exhaust.
[2]Average of 1000 cc min. vol. conditions under intermittent puffing.

Table 5-8

Comparison of Dose Offered

ADL Machine versus Simulated Animal Smoking

Per Cigarette Delivery

	TPM	Nicotine	Water	Tar	CO	CO_2	Acetaldehyde	Acrolein
	mg	mg	mg	mg	ml	ml	mg	mg
ADL	46	2.9	4.6	38.3	20.5	42.2	1.1	0.16
Dog	123	4.9	37.5	81.5	32.4	95.5	2.6	0.31
Analytical	66	3.7	10.1	51.8	18.5	41.4	1.4	0.13
Percent of Dog Puffing								
ADL	37%	59	12	47	63	44	42	52

TABLES

Section 8:00

Table 8-1 Influence of Label and Labeling Technique on Dose (% of Input) in Nosebreathing and Cannulated Hamsters; Whole cigarette, ^{14}C-hexadecane (C_{16}) butt.

Table 8-2 (same page as 8-1) Influence of Label and Labeling Technique on Dose (% of Chamber Input) in Nosebreathing and Cannulated Hamsters; Whole cigarette, ^{14}C-dotriacontane (C_{32}) throughout.

Table 8-3 Influence of Label and Labeling Technique on Dose (% of Input); ^{14}C-dotriacontane (C_{32}) Throughout Cigarette, Single Puff, Immediate Sacrifice.

Table 8-4 Respiratory Parameters in the Syrian Golden Hamster; Nosebreathing versus Cannulated.

Table 8-5 (same page as 8-4) The Influence of Respiratory Parameters on Lung Deposition of Radiolabeled Cigarette Smoke.

Table 8-6 Nasal Filtration of Gas Phase Components of Cigarette Smoke.

Table 8-7 (same page as 8-6) Measurement of Blood CO H_b Levels in Hamsters After Exposure to Cigarette Smoke.

Table 8-1

Influence of Label and Labeling Technique on Dose (% of input) in Nosebreathing and Cannulated Hamsters

Whole cigarette, ^{14}C-hexadecane (C_{16}) butt:

Experiment Number	Lung Deposition	
	Nosebreathing	Cannulated
1	0.022	0.63
2	0.20	
3a	0.22	0.27
5	0.30	0.55
6	0.40	
8a	0.26	0.66
12	0.21	0.37
Average	0.26	0.50
Whole cigarette, ^{14}C-hexadecane (C_{16}) throughout:		
11	0.26	0.53

Table 8-2

Influence of Label and Labeling Technique on Dose (% of Chamber Input) in Nosebreathing and Cannulated Hamsters

Whole cigarette, ^{14}C-dotriacontane (C_{32}) throughout:

Experiment Number	Lung Deposition	
	Nosebreathing	Cannulated
13	0.60	0.98
21 A	0.42	0.48
Average	0.51	0.73

Table 8-3

Influence of Label and Labeling Technique on Dose (% of Input):

^{14}C-dotriacontane (C_{32}) Throughout Cigarette, Single Puff, Immediate Sacrifice

	Percent of Input								
Nosebreathers									
Experiment Number	Lung	Larynx	Head	Liver	Trachea	Esophagus	Stomach	Intestine	Total
23	0.44	0	0.57	0	0	0	0.11	0	1.18
24a	0.38	0	0.27	0	0	0	0.05	0	0.70
24b	0.55	0	0.25	0	0	0	0.16	0	0.95
25	0.51	0	0.31	0	0.06	0	0.14	0	0.98
Average	0.47	0	0.35	0	0.01	0	0.13	0	0.96
Cannulated									
Experiment Number									
23	1.57	0	0	0	0	0	0.08	0	1.64
24a	1.40	0	0.08	0	0	0	0.09	0	1.55
24b	1.14	0	0	0	0	0	0.09	0	1.23
Average	1.37	0	0.02	0	0	0	0.09	0	1.48

Body cannulated/nose-breather = 1.54

Lung cannulated/nose-breather = 2.7

Table 8-4

Respiratory Parameters in The Syrian Golden Hamster

Nosebreathing versus Cannulated

	Tidal Volume		Breaths/min.		Minute volume (cc/min)	
Animal #	Nose	Can.	Nose	Can.	Nose	Can.
1	1.39*	1.59	83	47	117	87
2	1.57	2.03	72	60	114	118
3	1.09	1.56	77	48	83	72
4	1.25	1.98	70	58	87	115
5	0.83	1.89	133	53	108	102
Average	1.23	1.81	87	53	101	99
% Change by can.	68% increase		61% increase		No change	

*Each value = the mean of 3 measurements, with each measurement being done on a different day.

Table 8-5

The Influence of Respiratory Parameters on Lung Deposition of Radiolabeled Cigarette Smoke

	Tidal vol. (cc)	Breaths/min.	Minute vol. cc/min.	Lung Deposition
Group I simulates cannulated	1.5	51	77	13.3×10^3 DPM*
Group II simulates nosebreathing	0.8	93	74	6.6×10^3 DPM*

*Mean value determined from measurement in 5 animals.

Table 8-6

Nasal Filtration of Gas Phase Components of Cigarette Smoke

	Acetaldehyde (μg)		Acrolein (μg)	
Control I	15	15.5	0.7	0.7
Control II	16		0.7	
Hamster I	10	9	0.4	0.35
Hamster II	8		0.3	
% Decrease by nose	58		50	

Table 8-7

Measurement of Blood CO H_b Levels in Hamsters After Exposure to Cigarette Smoke

No. cig.	Mins. of smoke	Avg. % CO-H_b
1	5	10.5
2	10	22.3
5	25	35.0
10	50	49.3

ORNL Intermittent Smoker

FIGURES

Section 2:00

Figure 2-1 Six Port Sequential Smoking Machine Assembly.

Figure 2-2 Sample Averaging Smoking Machine Assembly. Top View.

Figure 2-3 Sample Averaging Smoking Machine Assembly. Bottom View.

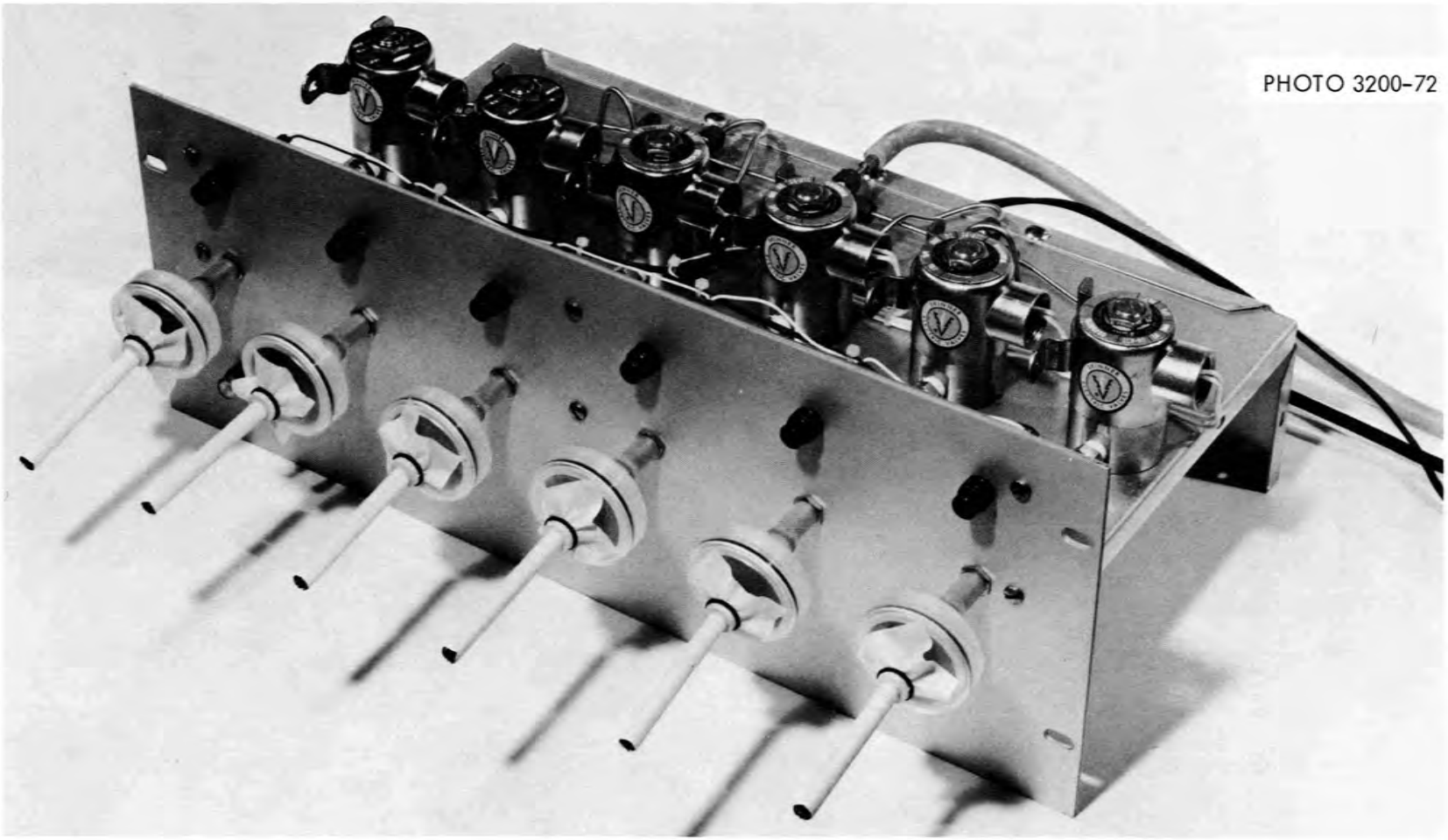

Figure 2-1 Six Port Sequential Smoking Machine Assembly.

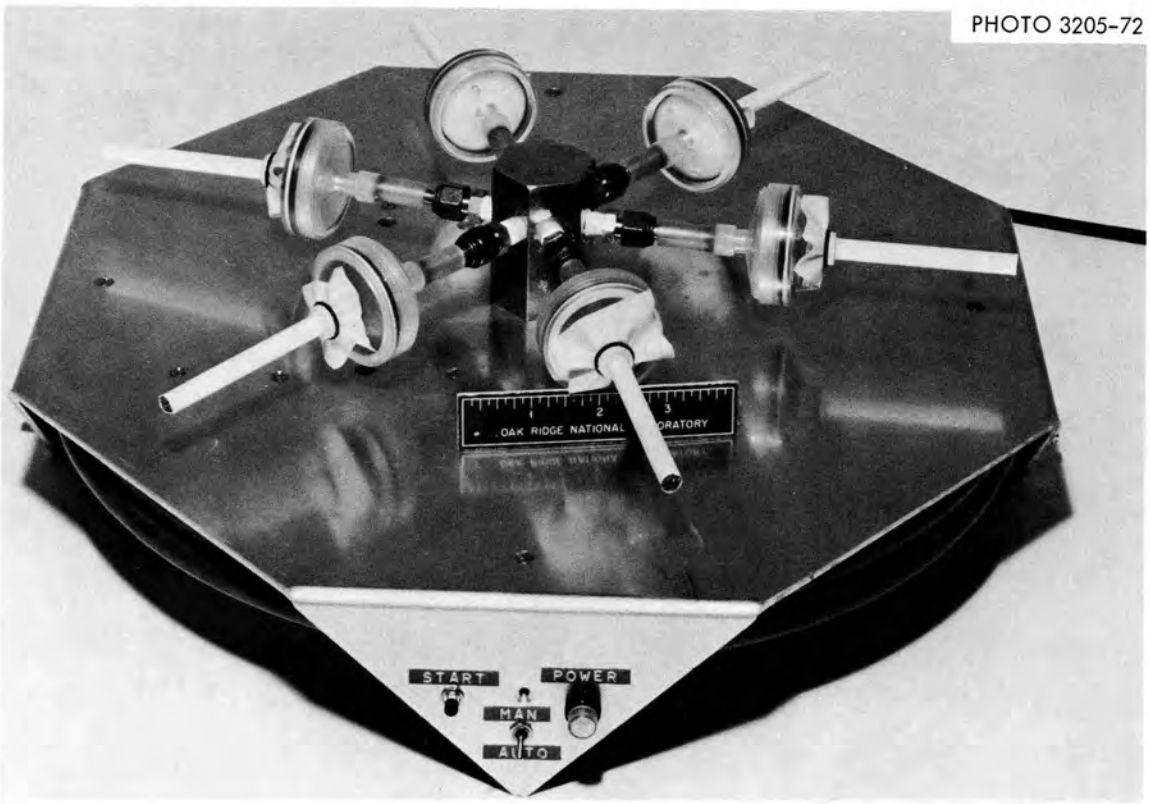

Figure 2-2 Sample Averaging Smoking Machine Assembly. Top View.

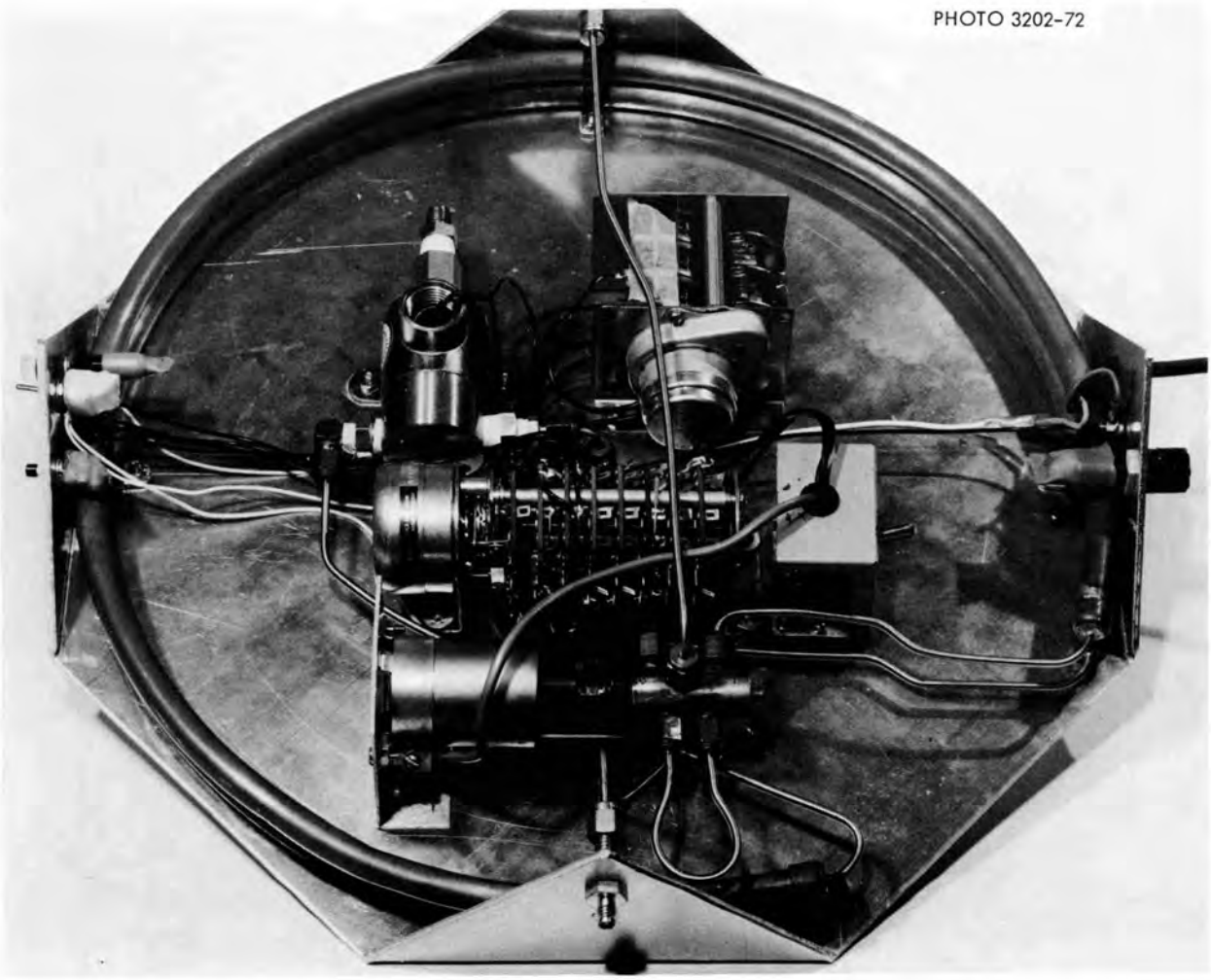

Figure 2-3 Sample Averaging Smoking Machine Assembly. Bottom View.

FIGURES

Section 3:00

Figure 3-1 Apparatus for the Preparation of Acetaldehyde and Acrolein Standards for the Cryothermal Gas Chromatography of Smoke Gas Phase.

Figure 3-2 Separation of Carbon Monoxide and Carbon Dioxide on a Single Column.

Figure 3-3 Hydrogen Cyanide - Gas Phase versus Whole Smoke Procedure.

Figure 3-4 Simultaneous Gas Chromatographic Analysis of Nicotine and Phenol plus Cresols Using Two Independent Columns in a Single Instrument.

Figure 3-5 Isothermal Gas Chromatographic Separation of Smoke Phenols on Trixylol Phosphate.

Figure 3-6 Computer Formated Display of First Versus Second Analysis Results for per Cigarette Nicotine Deliveries of Experimental Cigarettes Numbers 1-23.

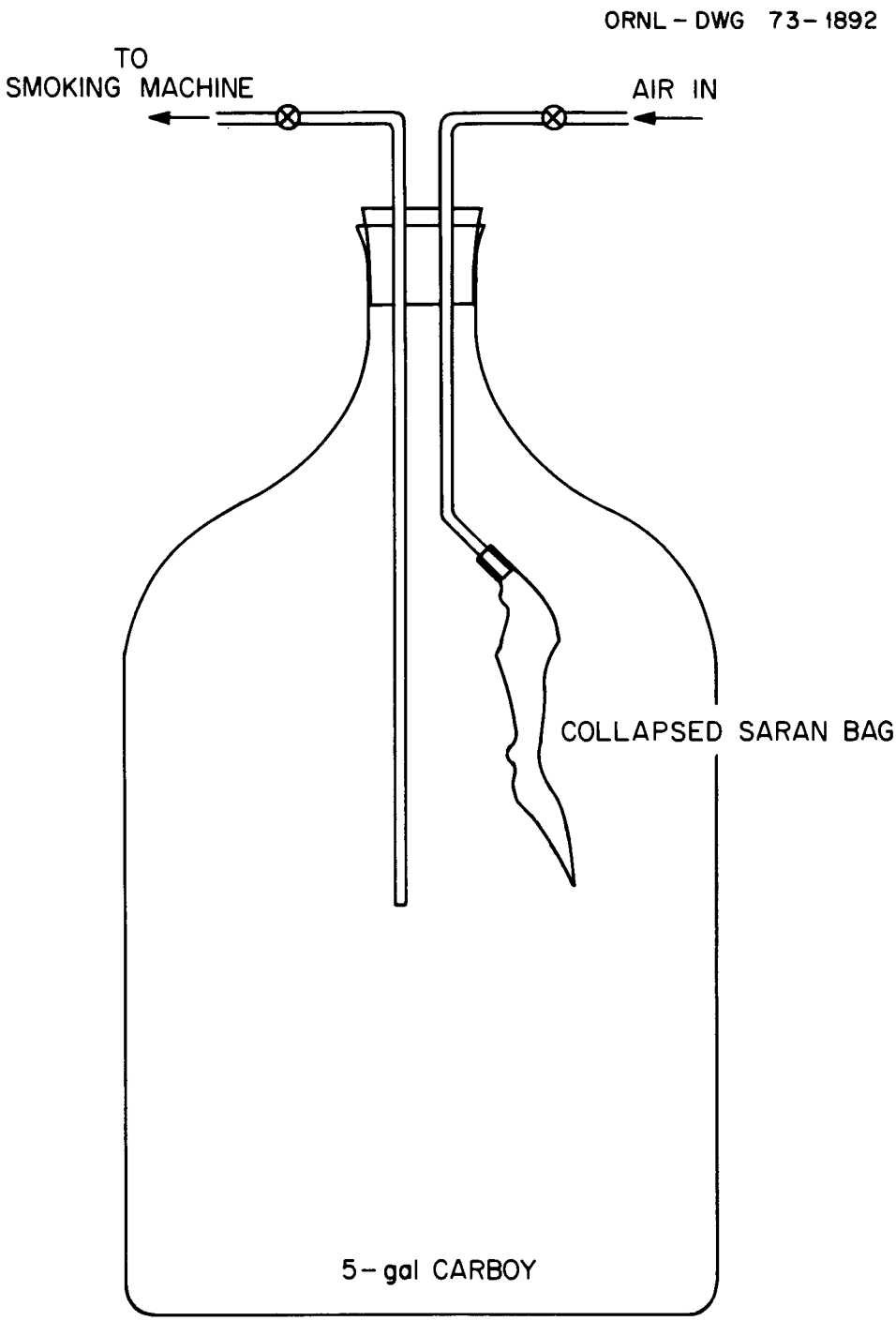

Figure 3-1 Apparatus for the Preparation of Acetaldehyde and Acrolein Standards for the Cryothermal Gas Chromatography of Smoke Gas Phase.

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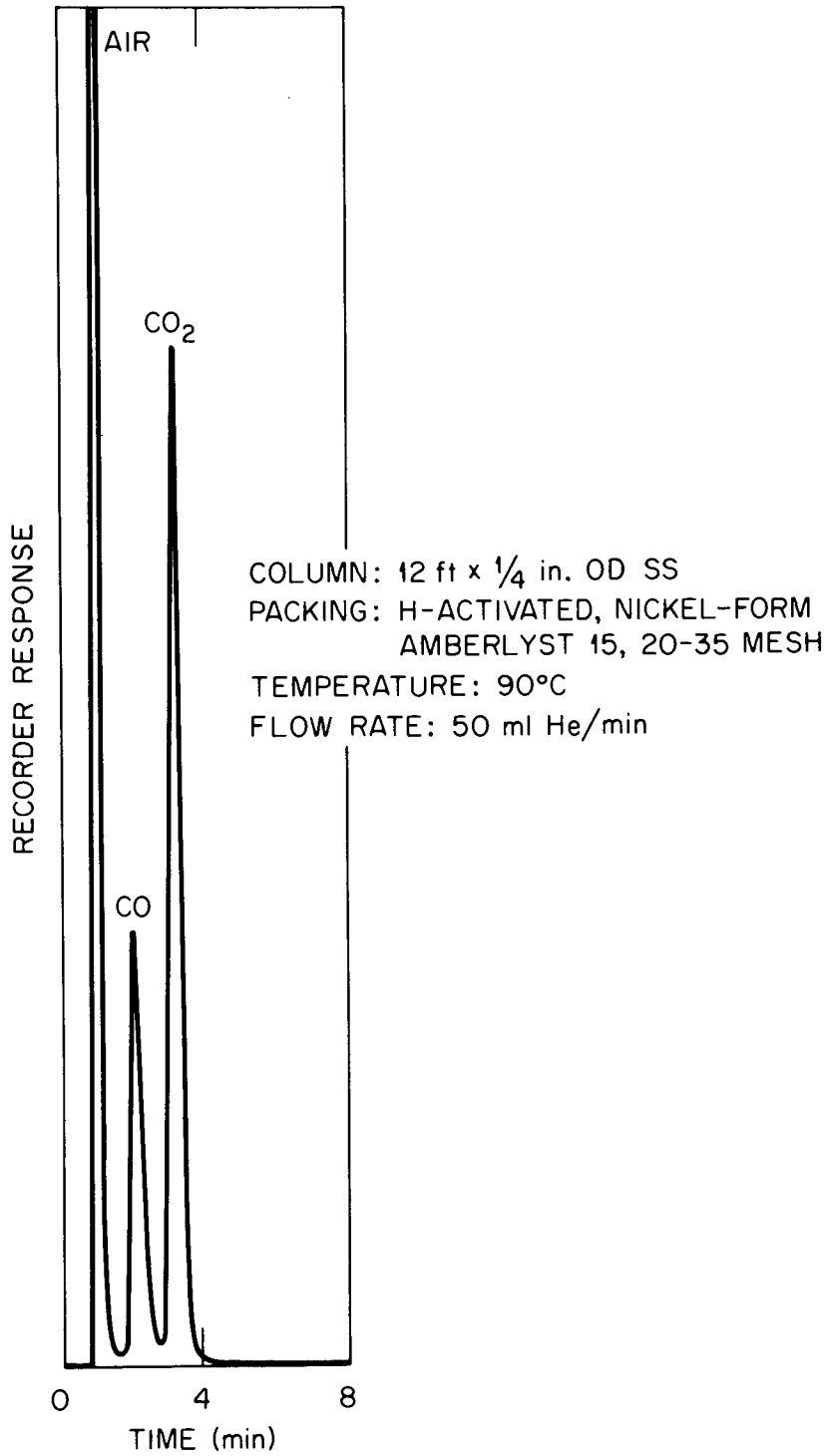

Figure 3-2 Separation of Carbon Monoxide and Carbon Dioxide on a Single Column.

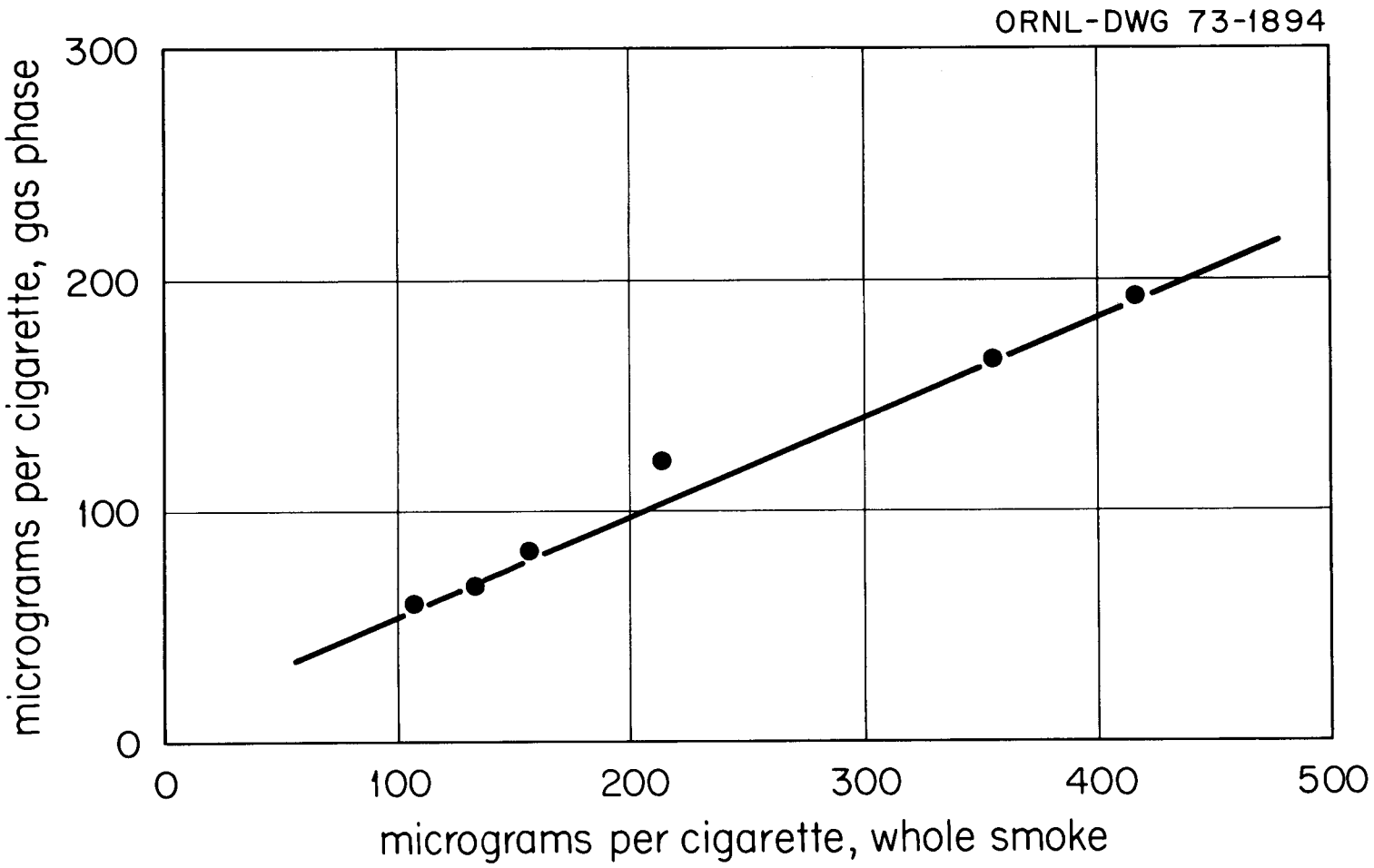

Figure 3-3 Hydrogen Cyanide - Gas Phase Versus Whole Smoke Procedure.

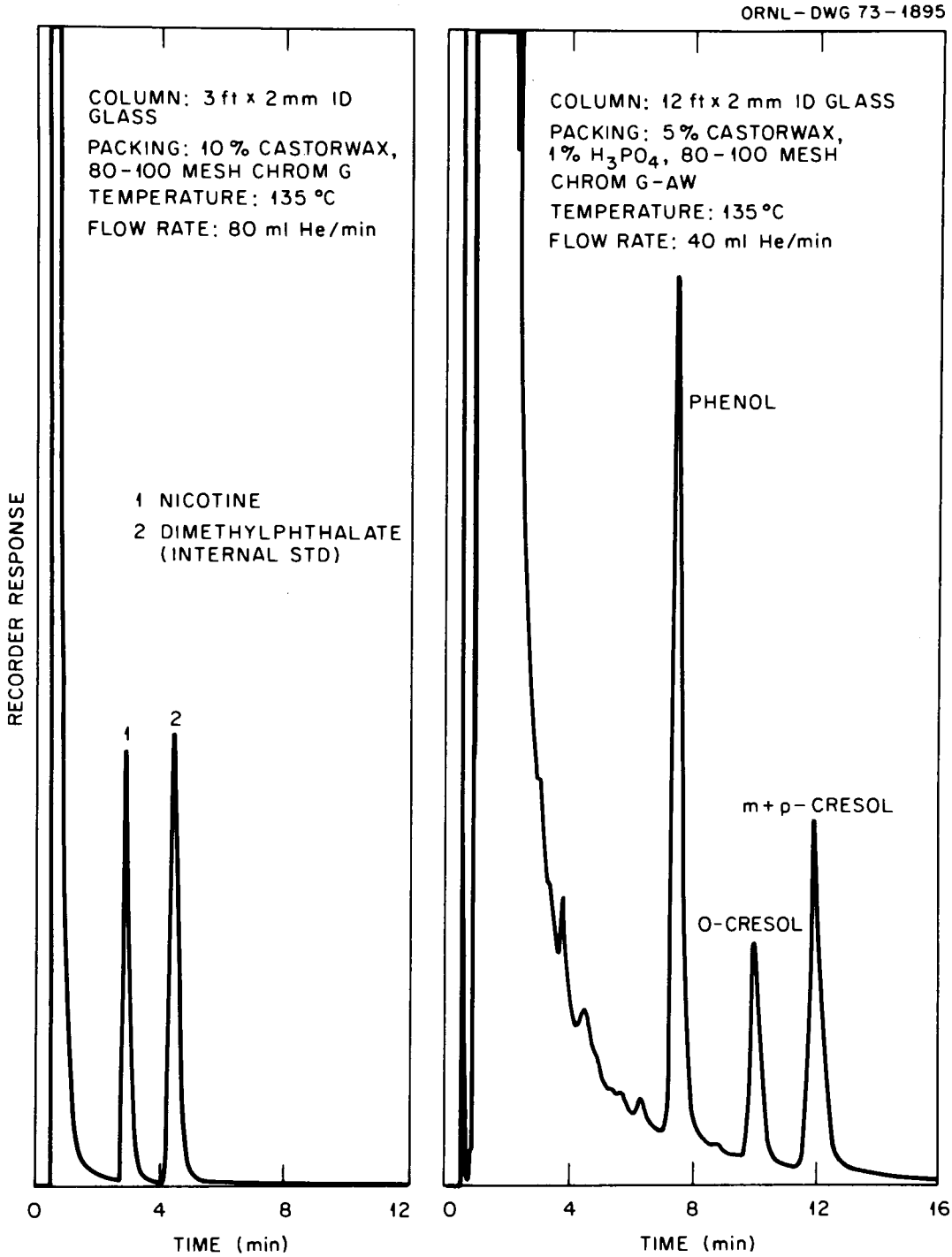

Figure 3-4 Simultaneous Gas Chromatographic Analysis of Nicotine and Phenol plus Cresols Using Two Independent Columns in a Single Instrument.

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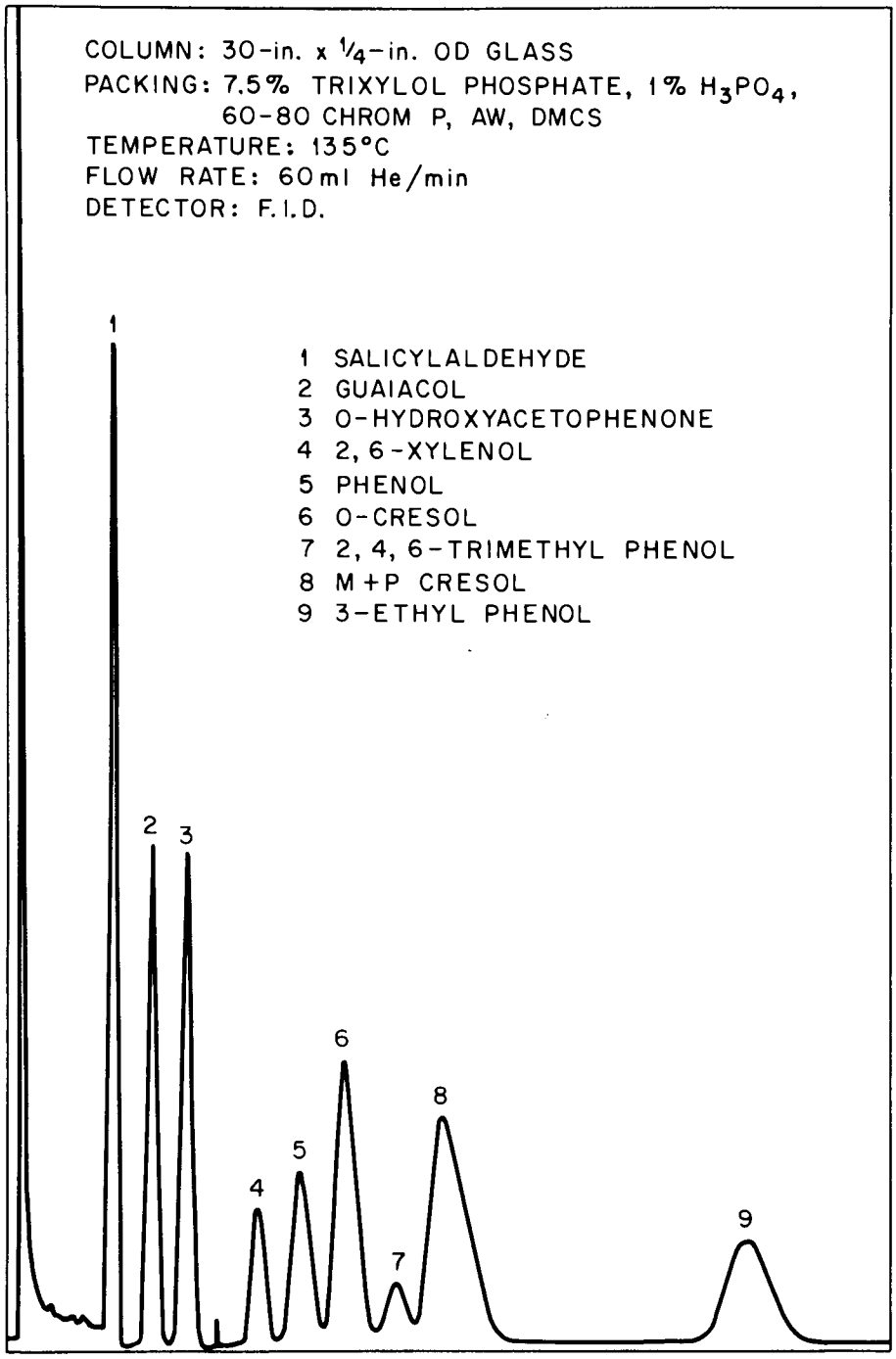


Figure 3-5 Isothermal Gas Chromatographic Separation of Smoke Phenols on Trixylol Phosphate.

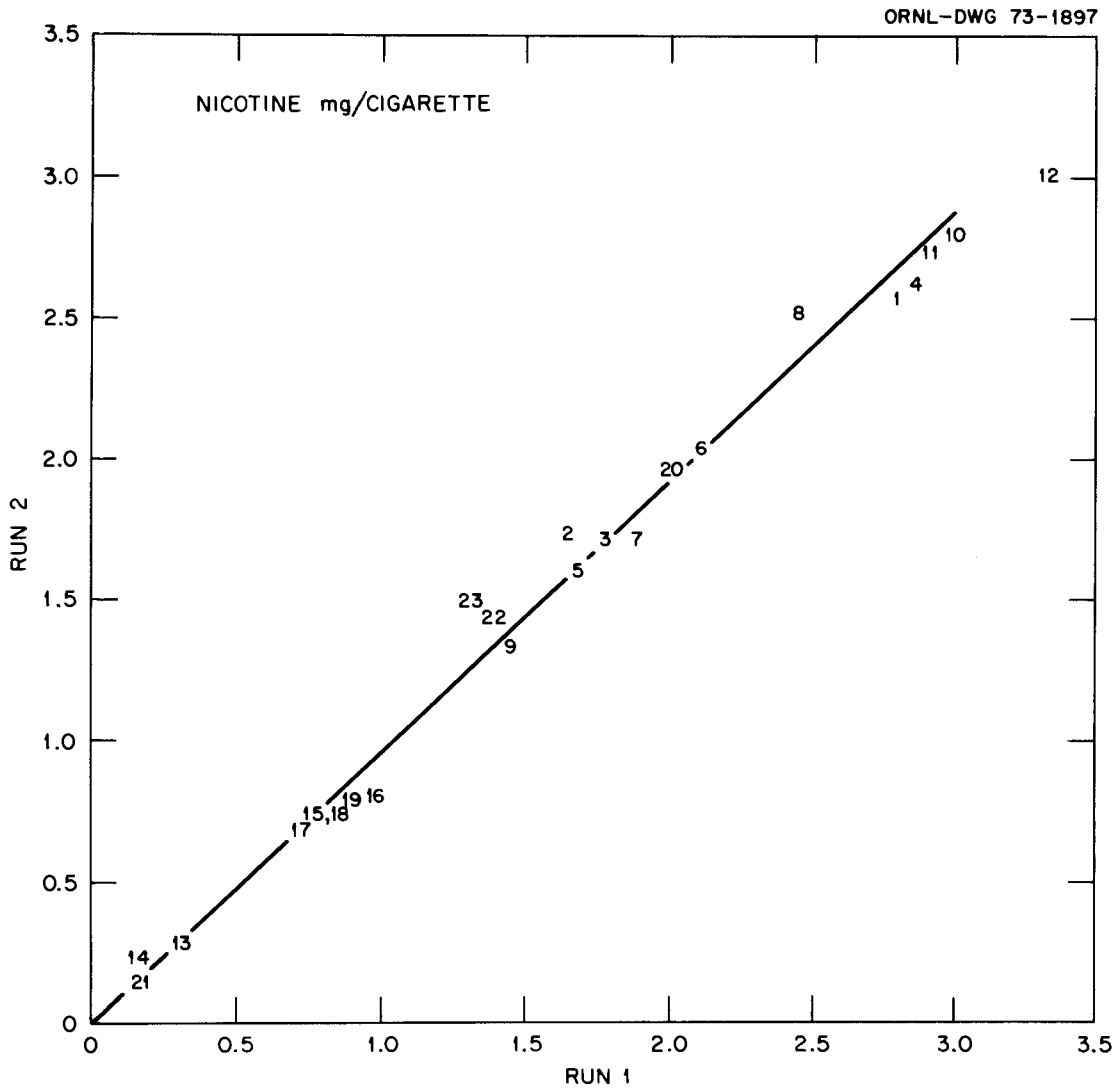


Figure 3-6 Computer Formated Display of First Versus Second Analysis Results for Per Cigarette Nicotine Deliveries of Experimental Cigarettes Number 1-23.

FIGURES

Section 4:00

Figure 4-1 Isothermal Separation of Palmitic (1), Oleic plus Linoleic plus Linolenic (2) and Stearic (3) Acids as Trimethylsilyl Esters.

Figure 4-2 Profile of Total Particulate Matter from a Commercial Filter Cigarette 18 ft. x 1/8 in. Column (sec. 4.13).

Figute 4-3 A Comparison of Total Particulate Matter Profiles for Smokes of Selected Cigarettes (sec. 4.13).

Figure 4-4 Profile of Total Particulate Matter from a 1R1 Reference Cigarette 6 ft. x 1/4 in. Column (sec. 4.13).

Figure 4-5 Single Port Smoking Machine in Use for Studies of Sulfur Compounds in Smokes.

Figure 4-6 Sulfur Containing Compounds in the Gas Phase of Smoke From a Kentucky Reference Cigarette (1R1).

Figure 4-7 Effect of Aging on Sulfur Compounds in the Gas Phase of Cigarette Smoke.

Figure 4-8 Sulfur Delivery from Puff to Puff and in the Course of a Single Puff (Dwg. 71-10594).

Figure 4-9 Profile of the Nitrogenous Components in the Gas Phase of a Single Puff of Smoke.

Section 4:00 Figures (continued)

Figure 4-10 Nitrogen-Selective Chromatographic Response to NO in the Gas Phase of a Single Puff of Smoke.

Figure 4-11 Sulfur Compounds in Phenanthrene Fraction of Cigarette Smoke Condensate.

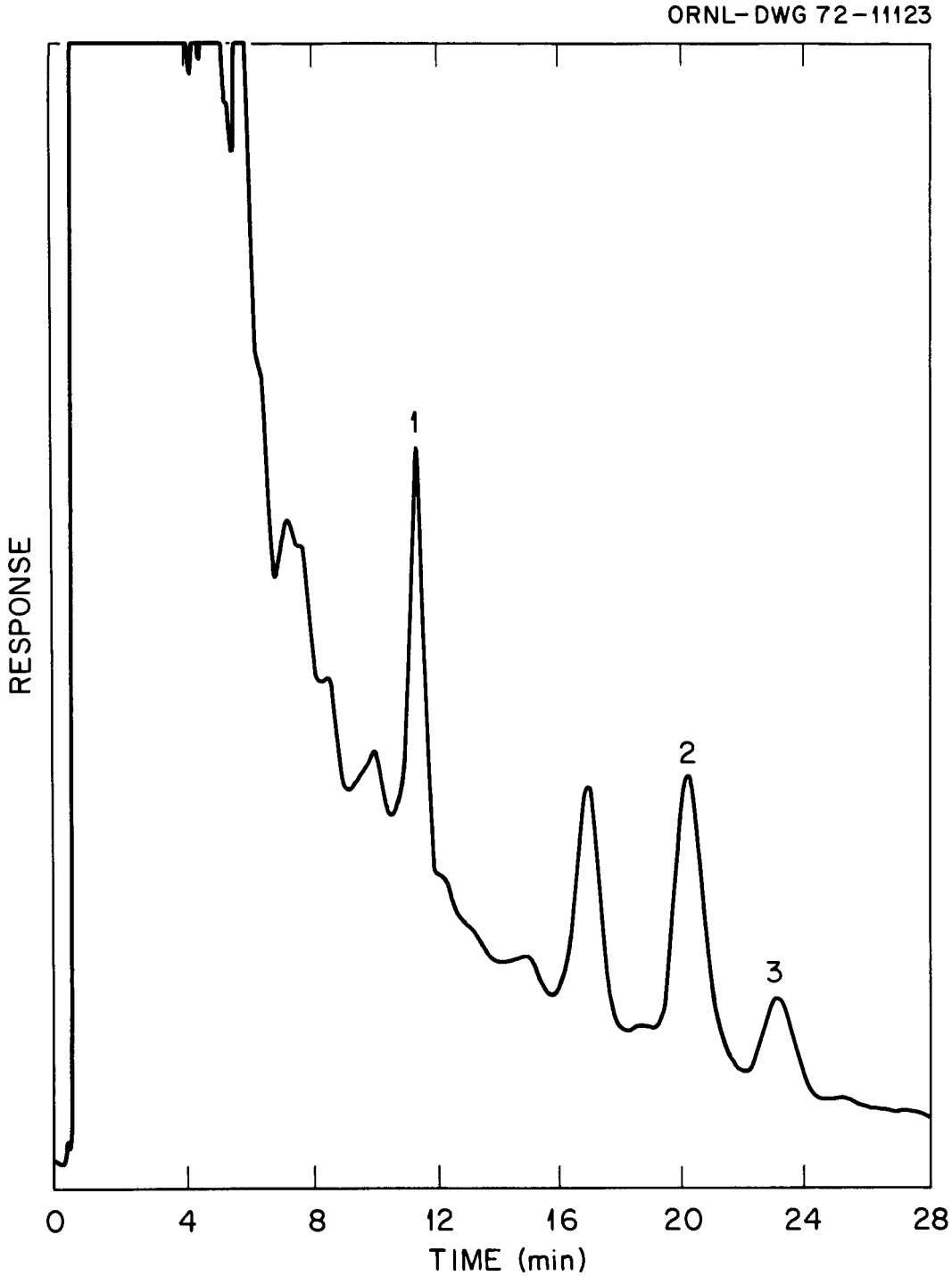


Figure 4-1 Isothermal Separation of Palmitic (1), Oleic plus Linoleic plus Linolenic (2) and Stearic (3) Acids as Trimethylsilyl Esters.

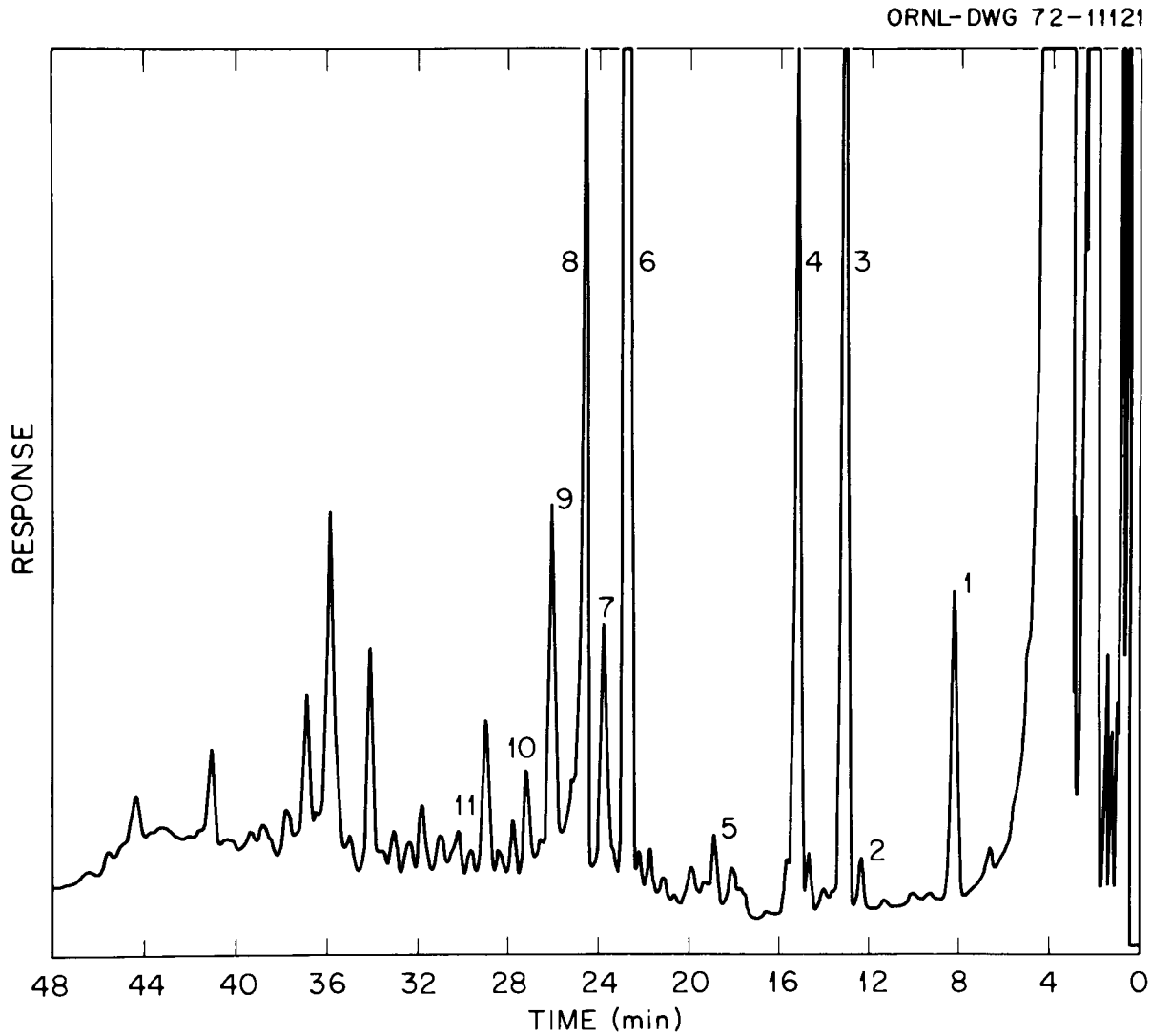


Figure 4-2 Profile of Total Particulate Matter from a Commercial Filter Cigarette 18 ft. x 1/8 in. Column (sec. 4.13).

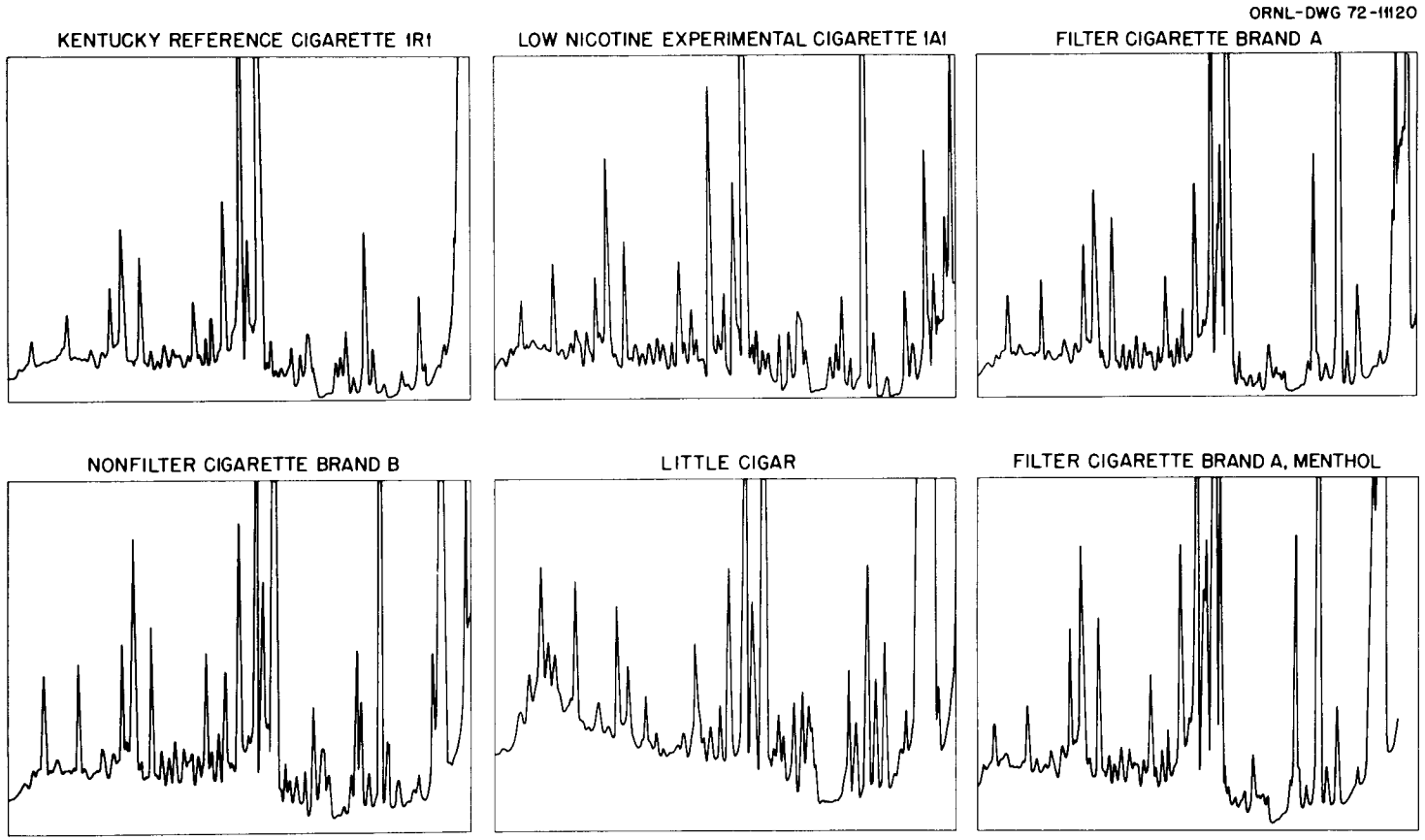


Figure 4-3 A Comparison of Total Particulate Matter Profiles for Smokes of Selected Cigarettes (sec. 4.13).

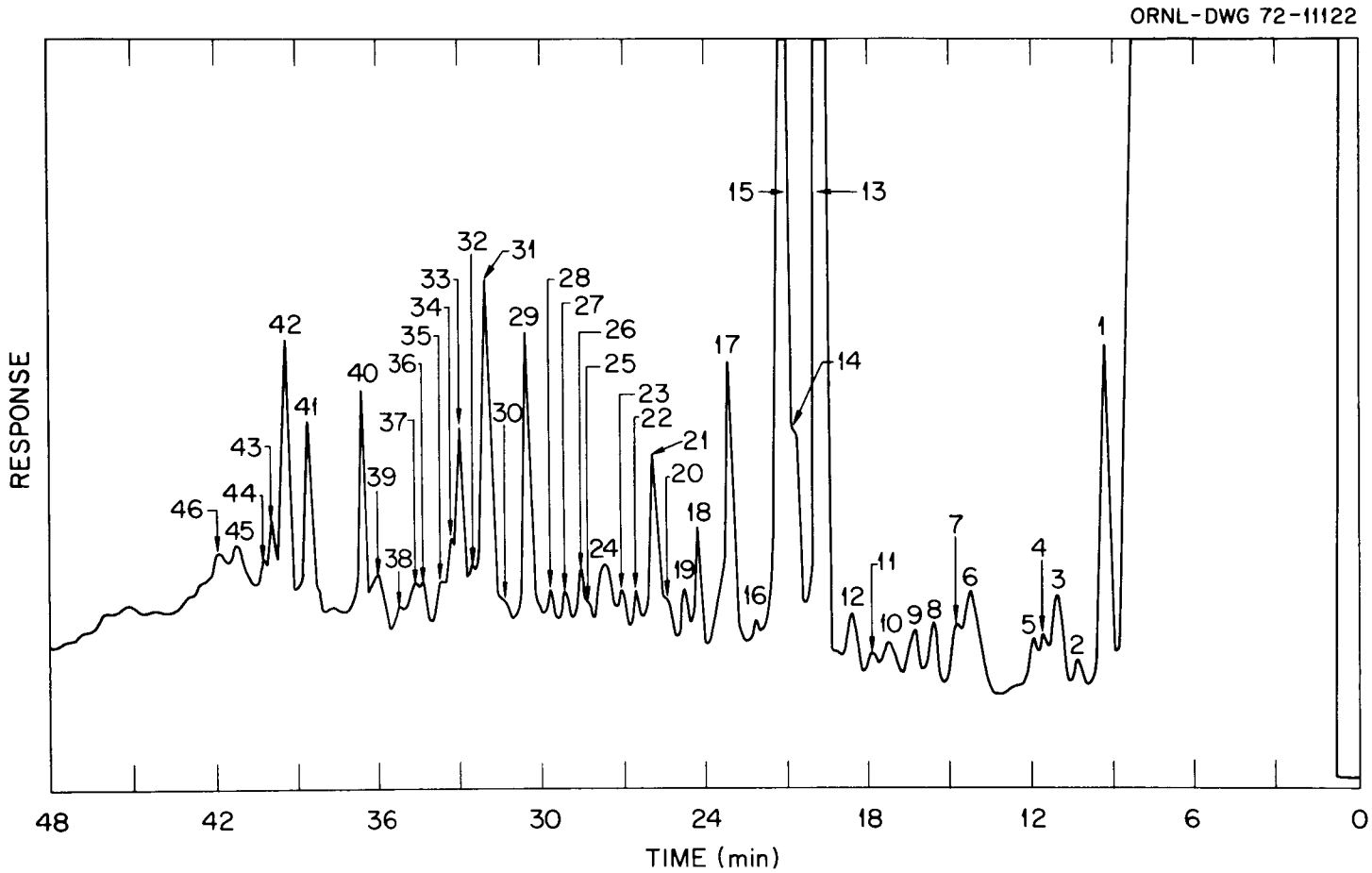


Figure 4-4 Profile of Total Particulate Matter from a 1R1 Reference Cigarette 6 ft. x 1/4 in. Column (sec. 4.13).

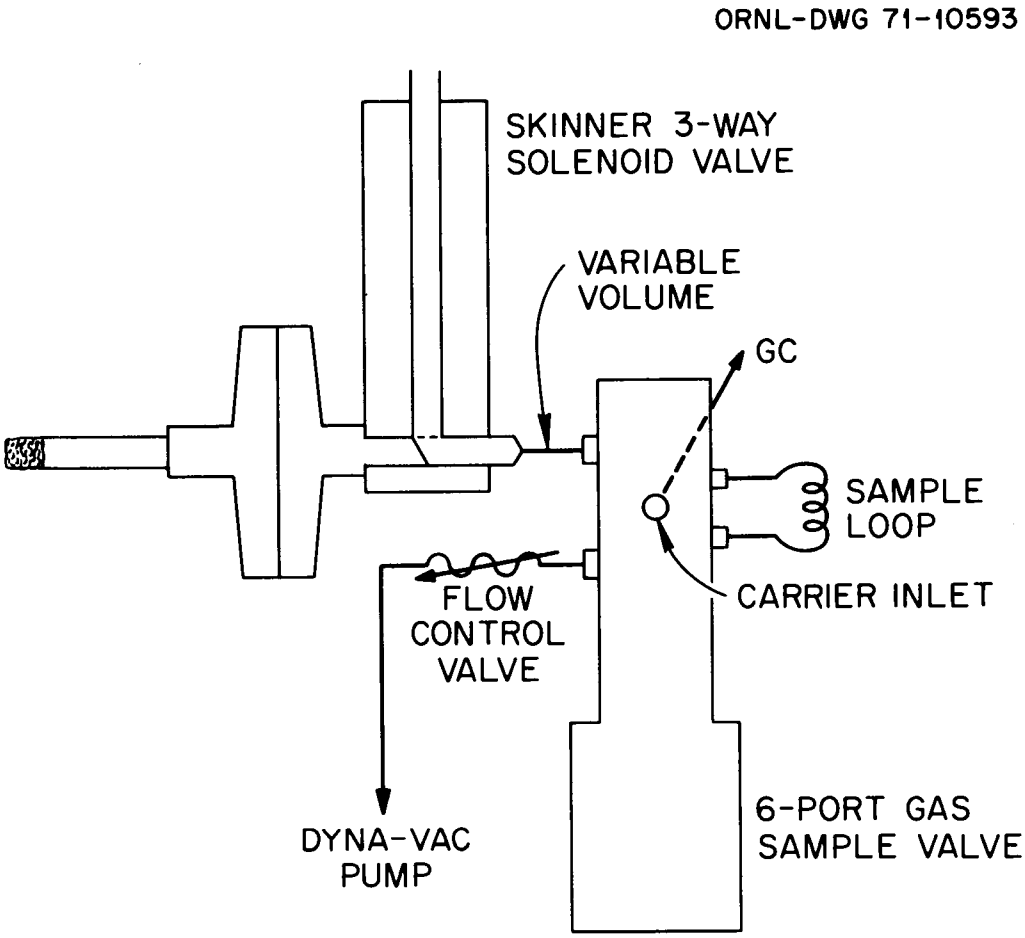


Figure 4-5 Single Port Smoking Machine in Use for Studies of Sulfur Compounds in Smokes.

ORNL-DWG 72-13597R

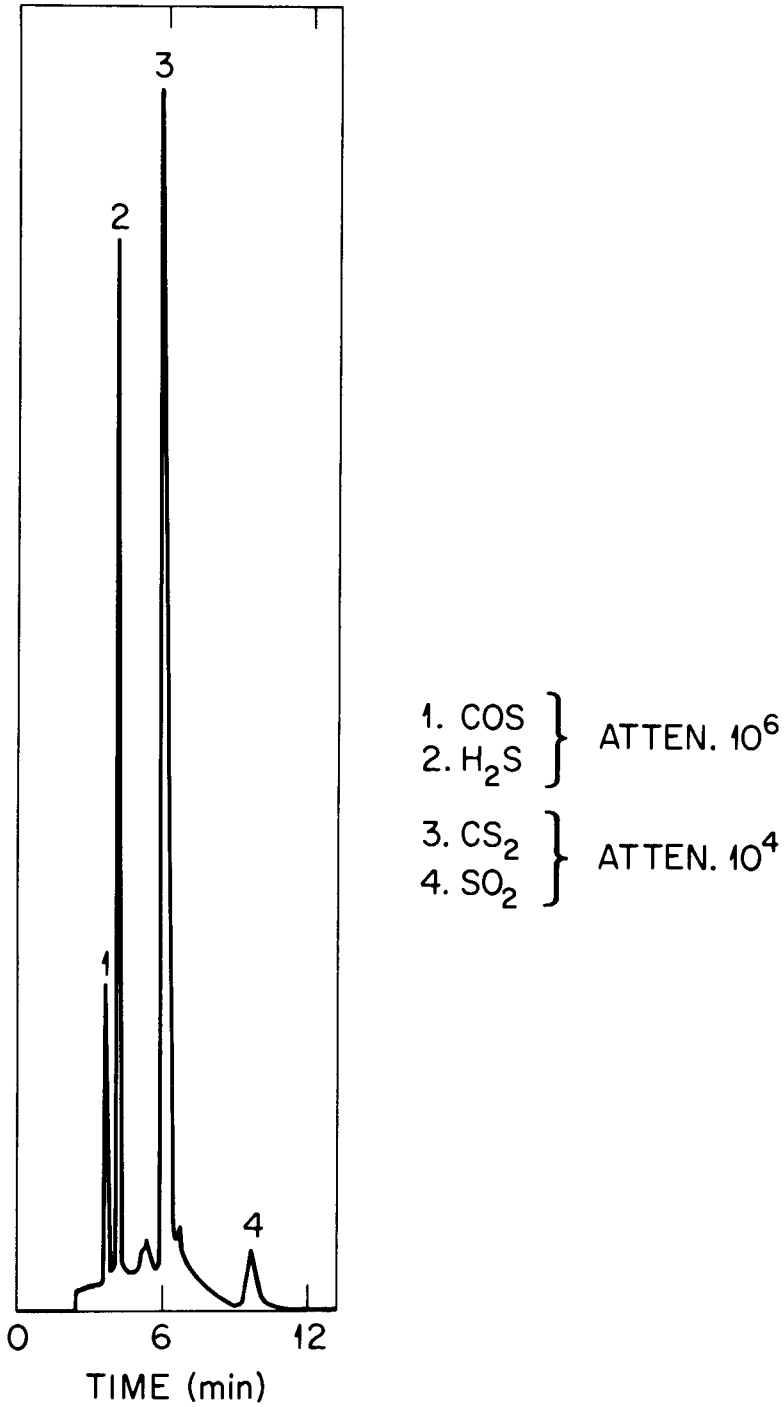


Figure 4-6 Sulfur Containing Compounds in the Gas Phase of Smoke from a Kentucky Reference Cigarette (1R1).

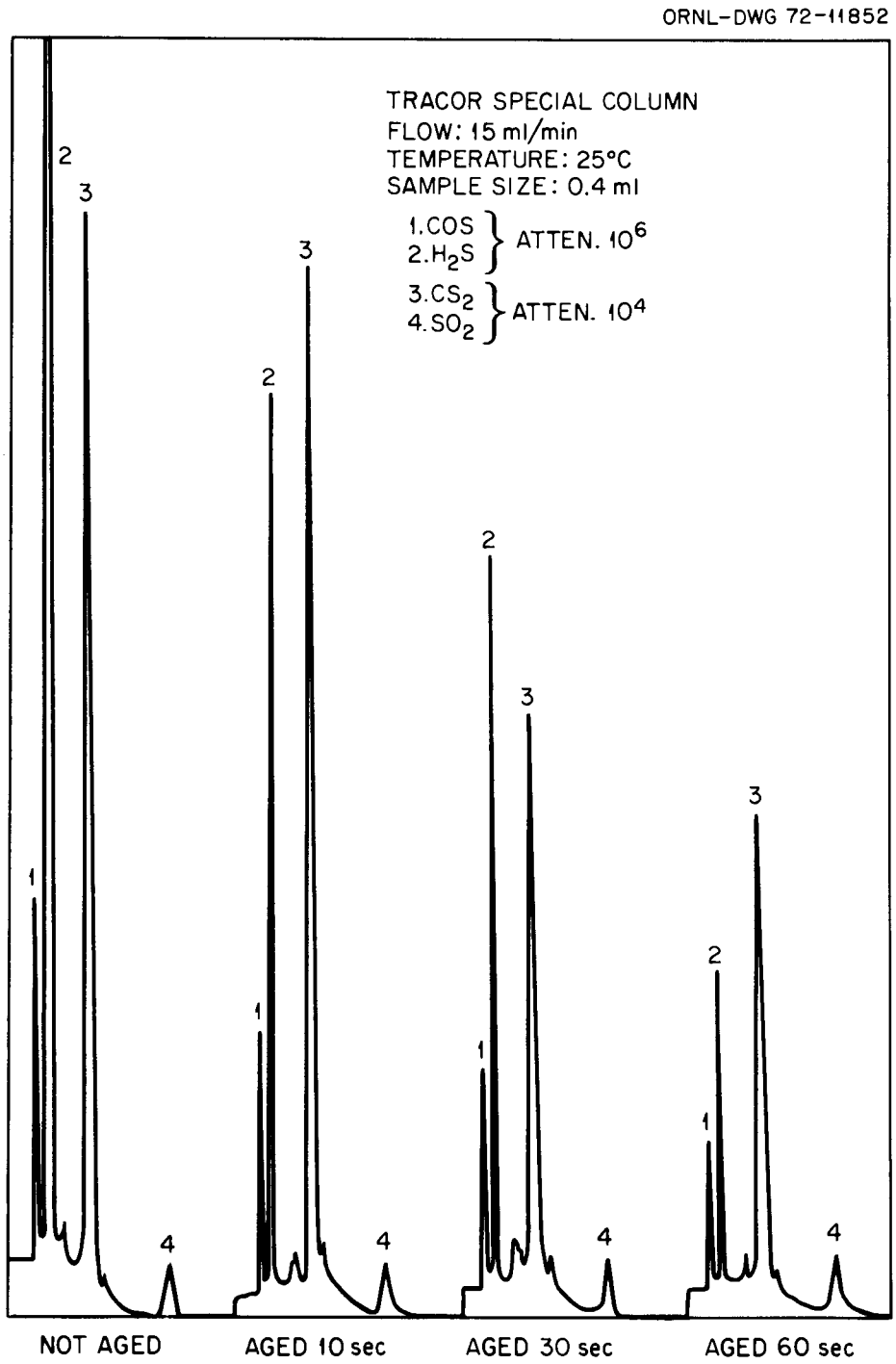


Figure 4-7 Effect of Aging on Sulfur Compounds in the Gas Phase of Cigarette Smoke.

ORNL-DWG 71-10594

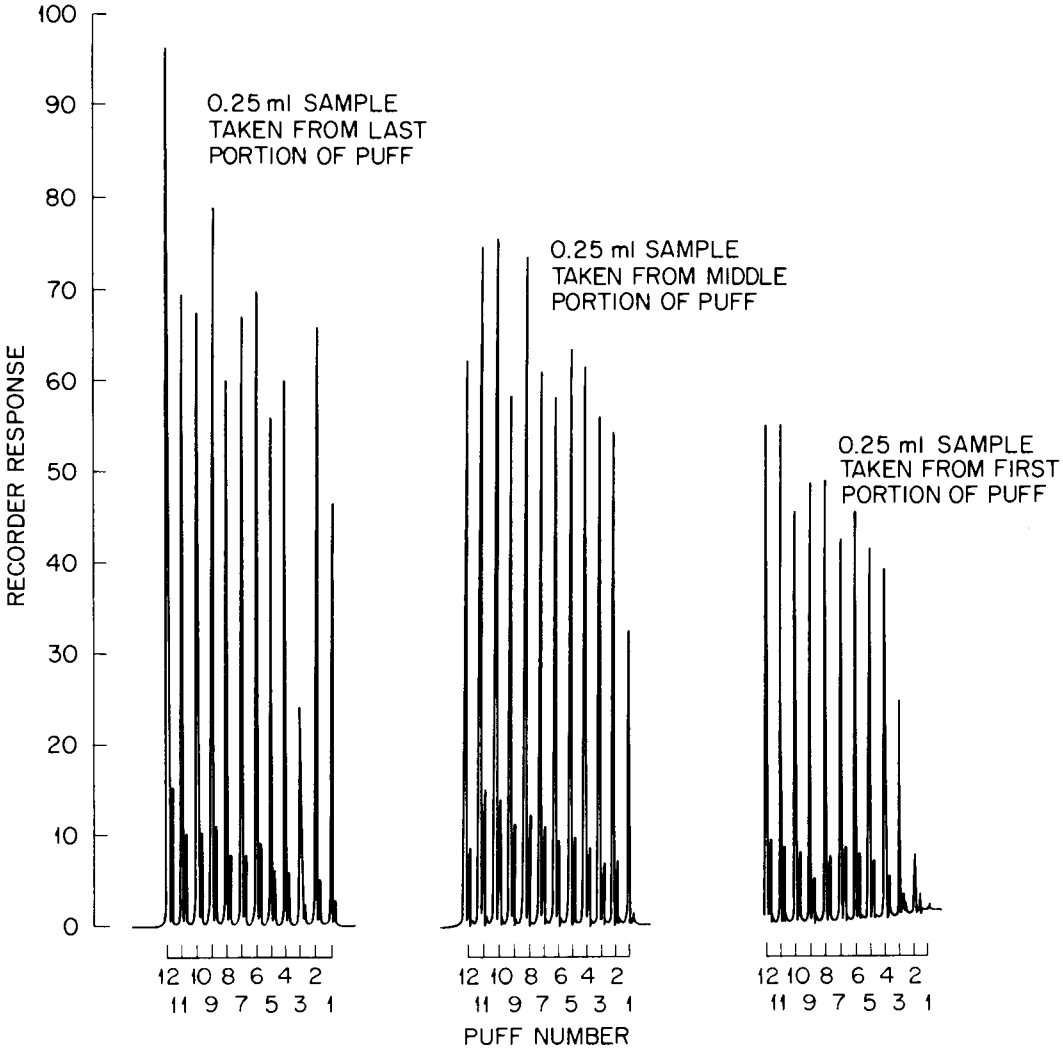


Figure 4-8 Sulfur Delivery from Puff to Puff and in the Course of a Single Puff.

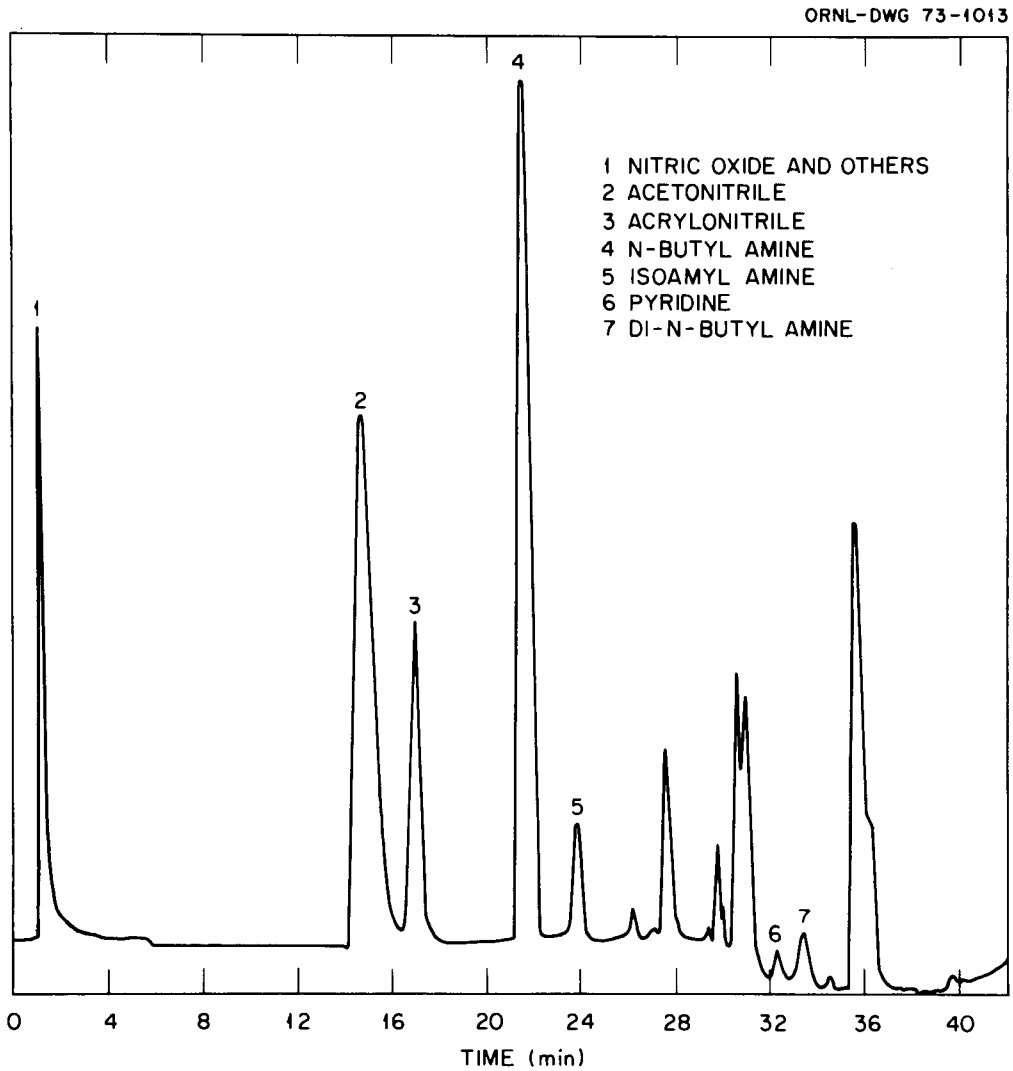


Figure 4-9 Profile of the Nitrogenous Components in the Gas Phase of a Single Puff of Smoke.

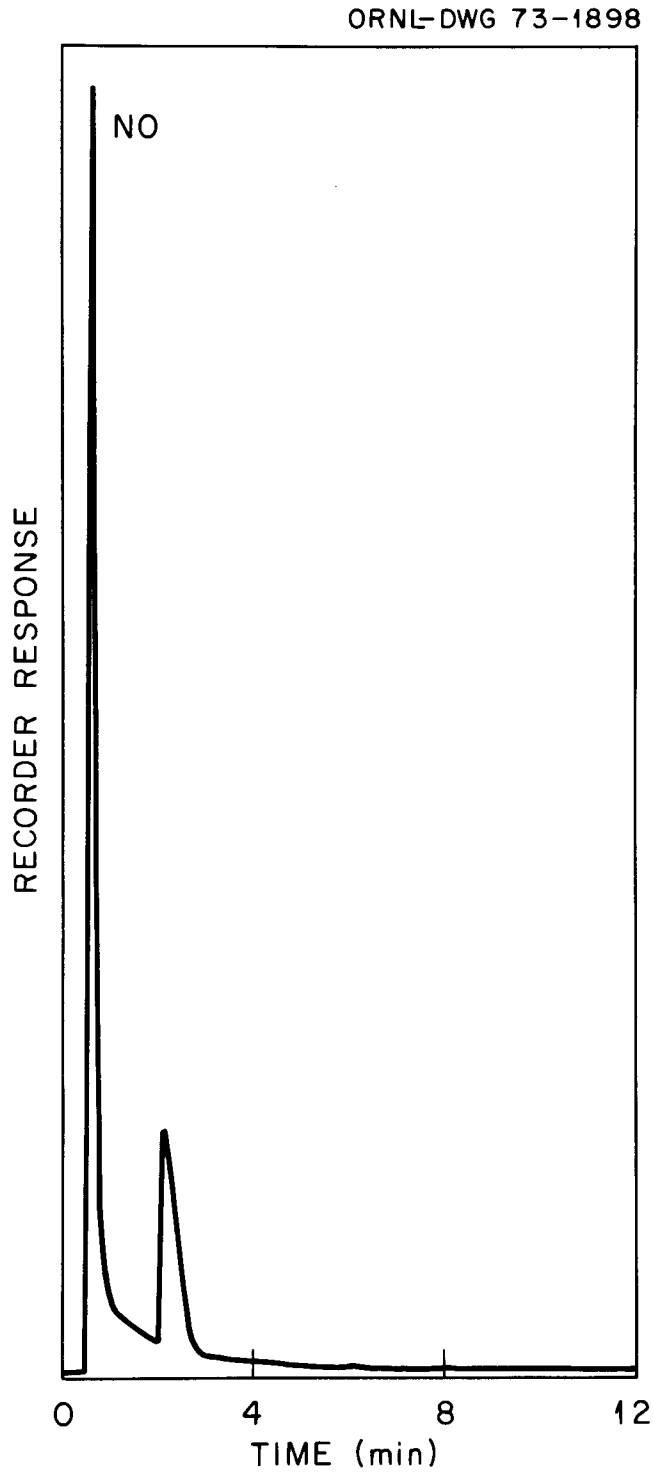


Figure 4-10 Nitrogen-Selective Chromatographic Response to NO in the Gas Phase of a Single Puff of Smoke.

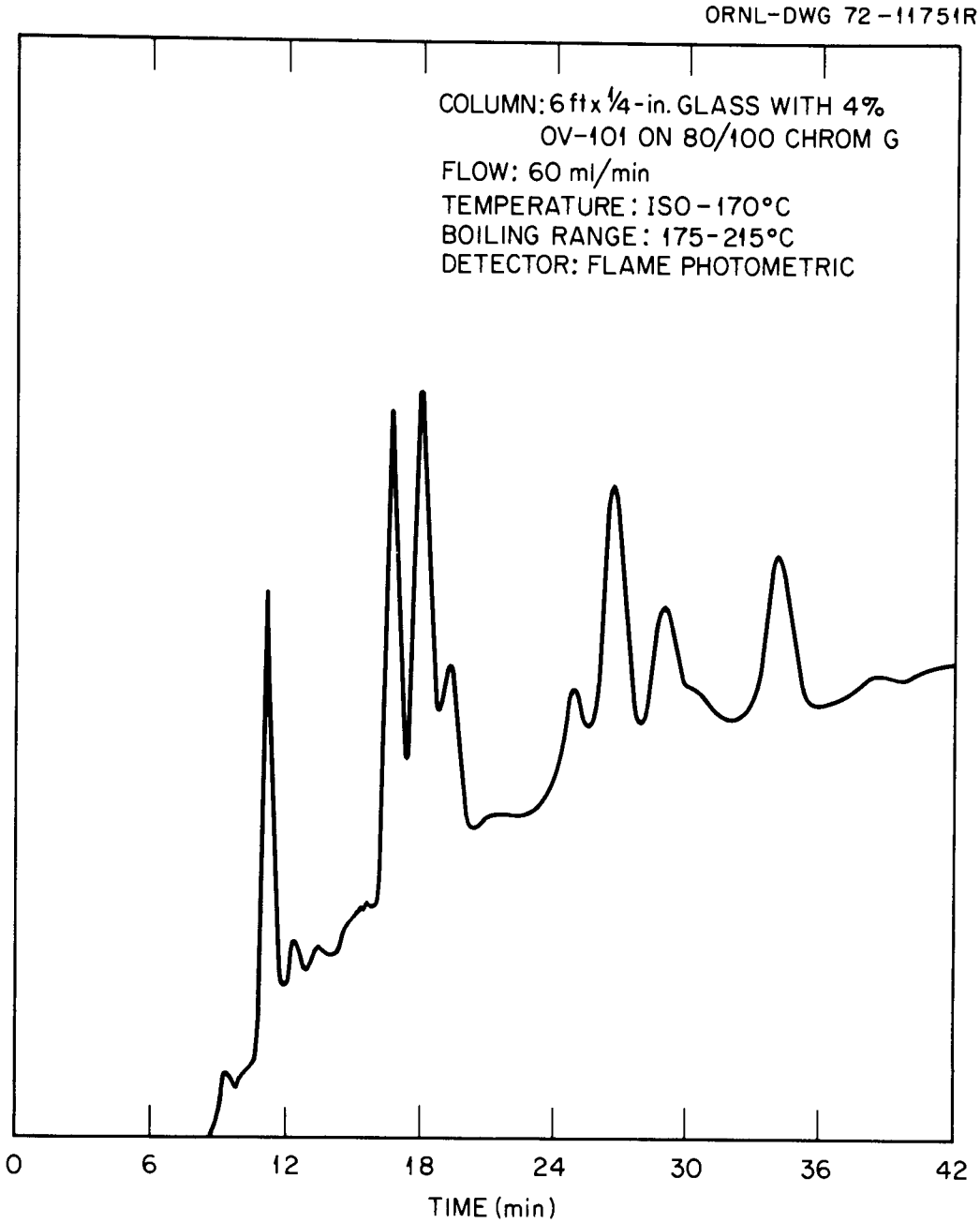


Figure 4-11 Sulfur Compounds in Phenanthrene Fraction of Cigarette Smoke Condensate.

FIGURES

Section 5:00

Figure 5-1 Gas Phase and TPM Profiles From Marijuana and Tobacco Cigarette Smokes.

Figure 5-2 Automated Equipment Developed by ADL for Chronically Exposing Dogs to Cigarette Smoke (courtesy S. P. Battista, Arthur D. Little, Inc., Cambridge, Massachusetts).

Figure 5-3 Profiles of TPM - ADL Machine Position, Analytically Generated Smoke and Simulated Dog Smoking (sec. 5.4a).

Figure 5-4 Profiles of Gas Phase - ADL Machine Position, Analytically Generated Smoke and Simulated Dog Smoking (sec. 5.4c).

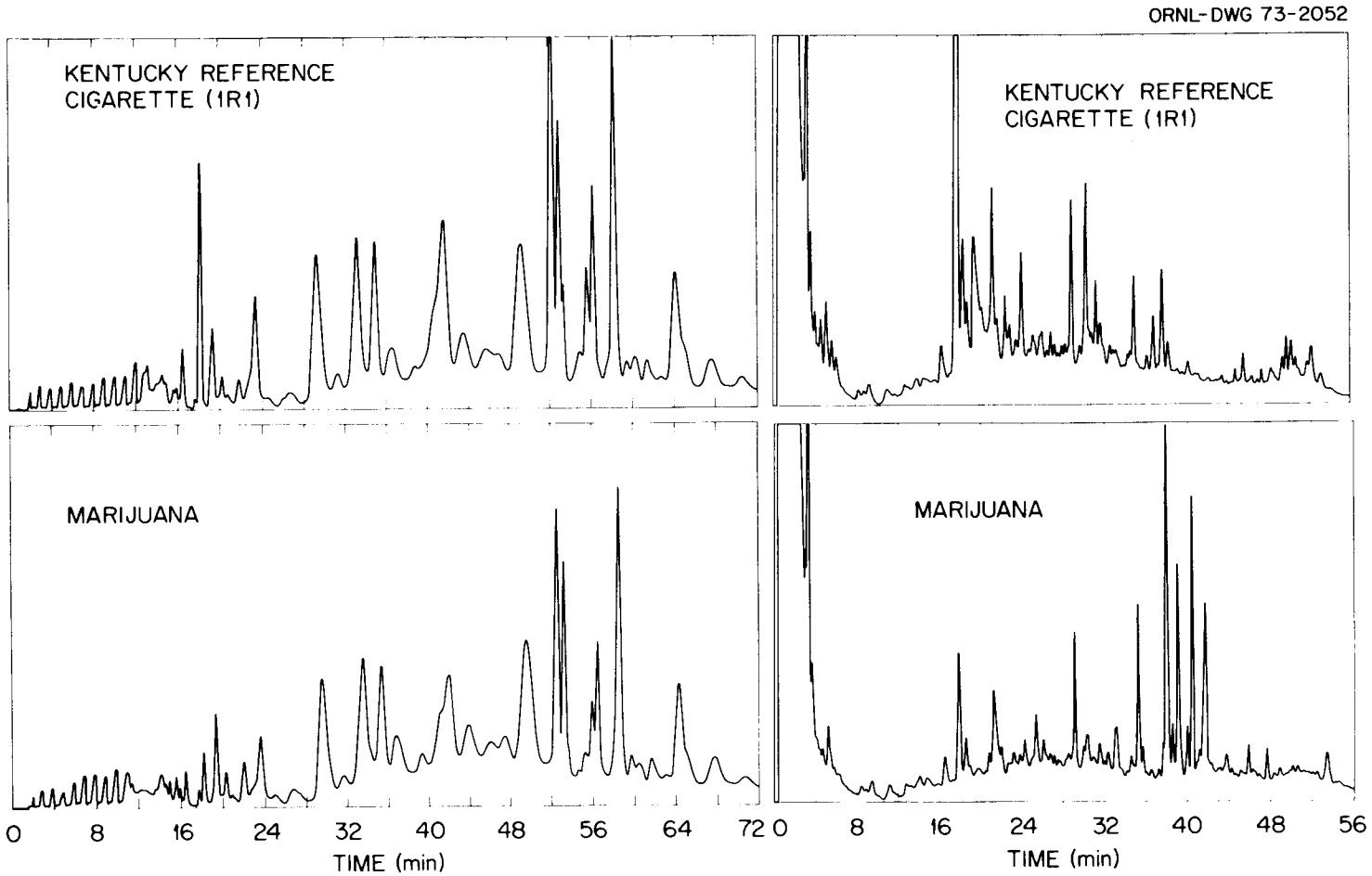
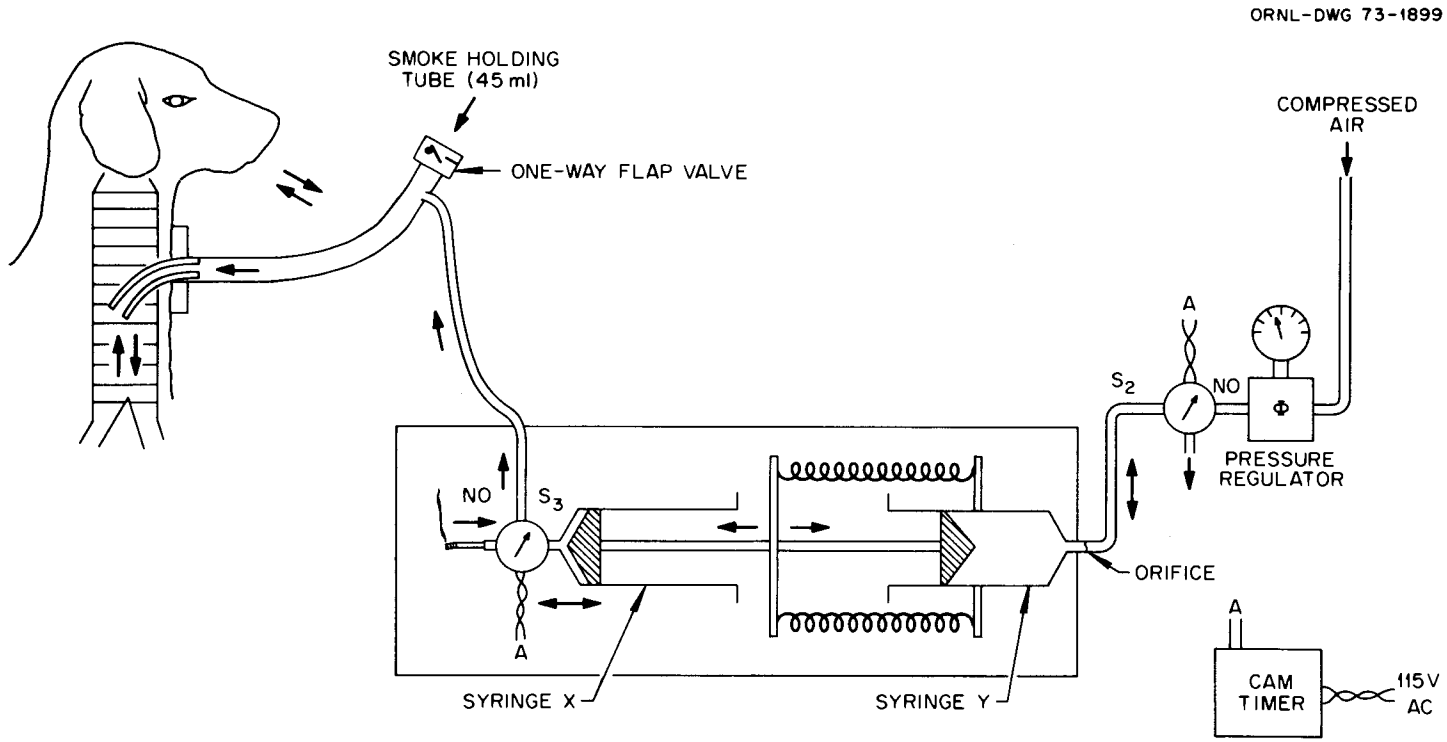


Figure 5-1 Gas Phase and TPM Profiles From Marijuana and Tobacco Cigarette Smokes.



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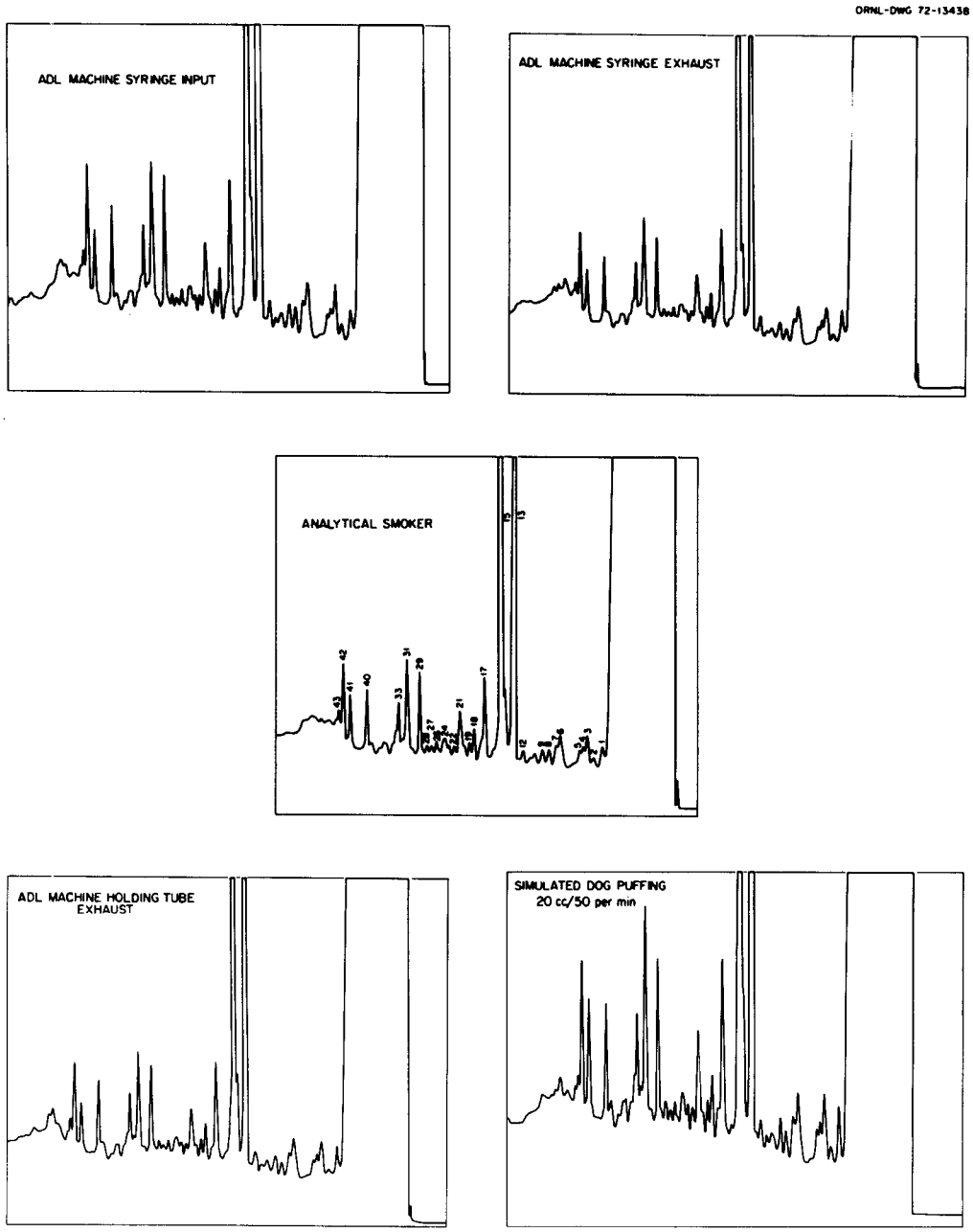


Figure 5-3 Profiles of TPM - ADL Machine Position, Analytically Generated Smoke and Simulated Dog Smoking (sec. 5.4a).

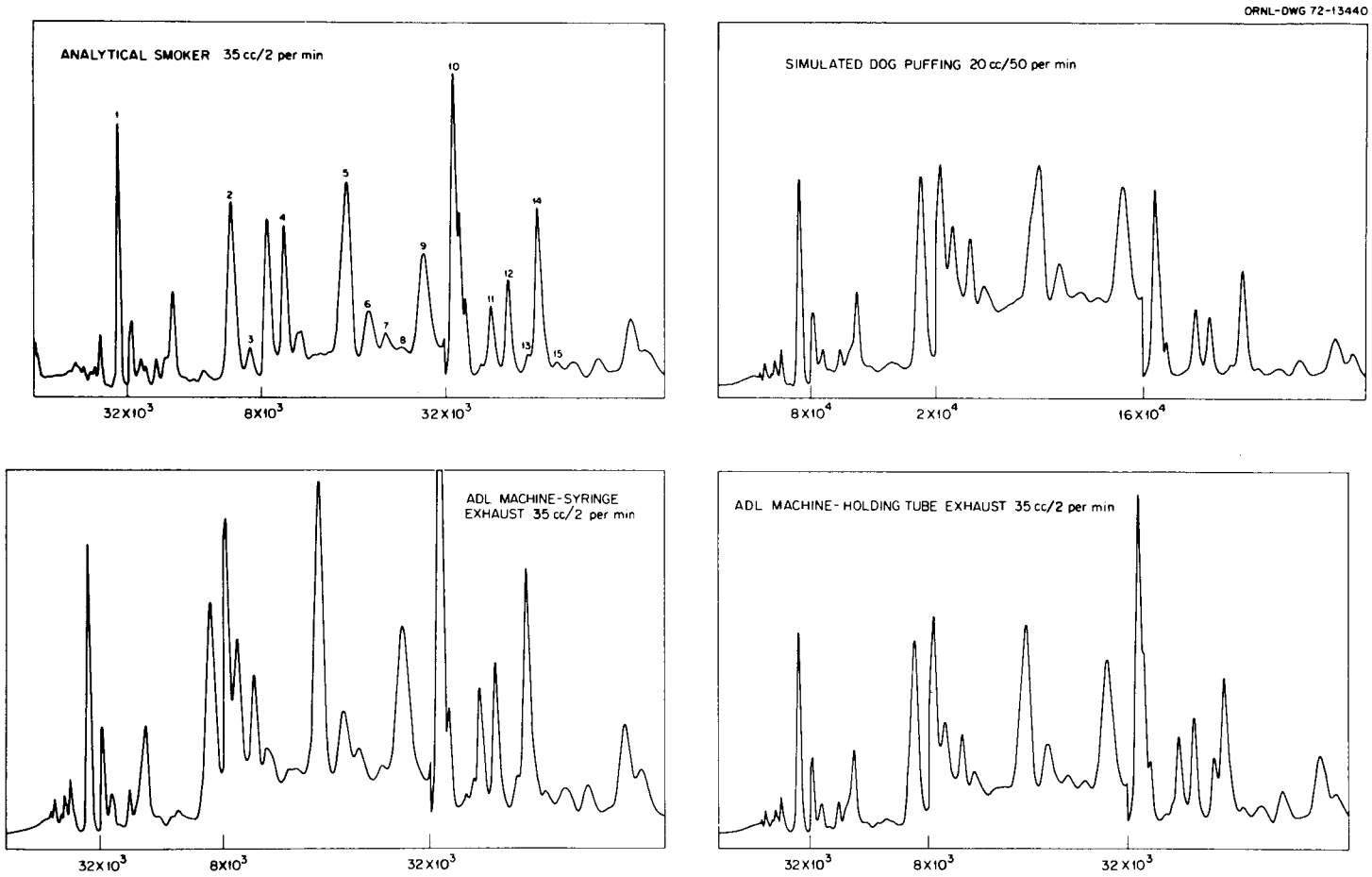


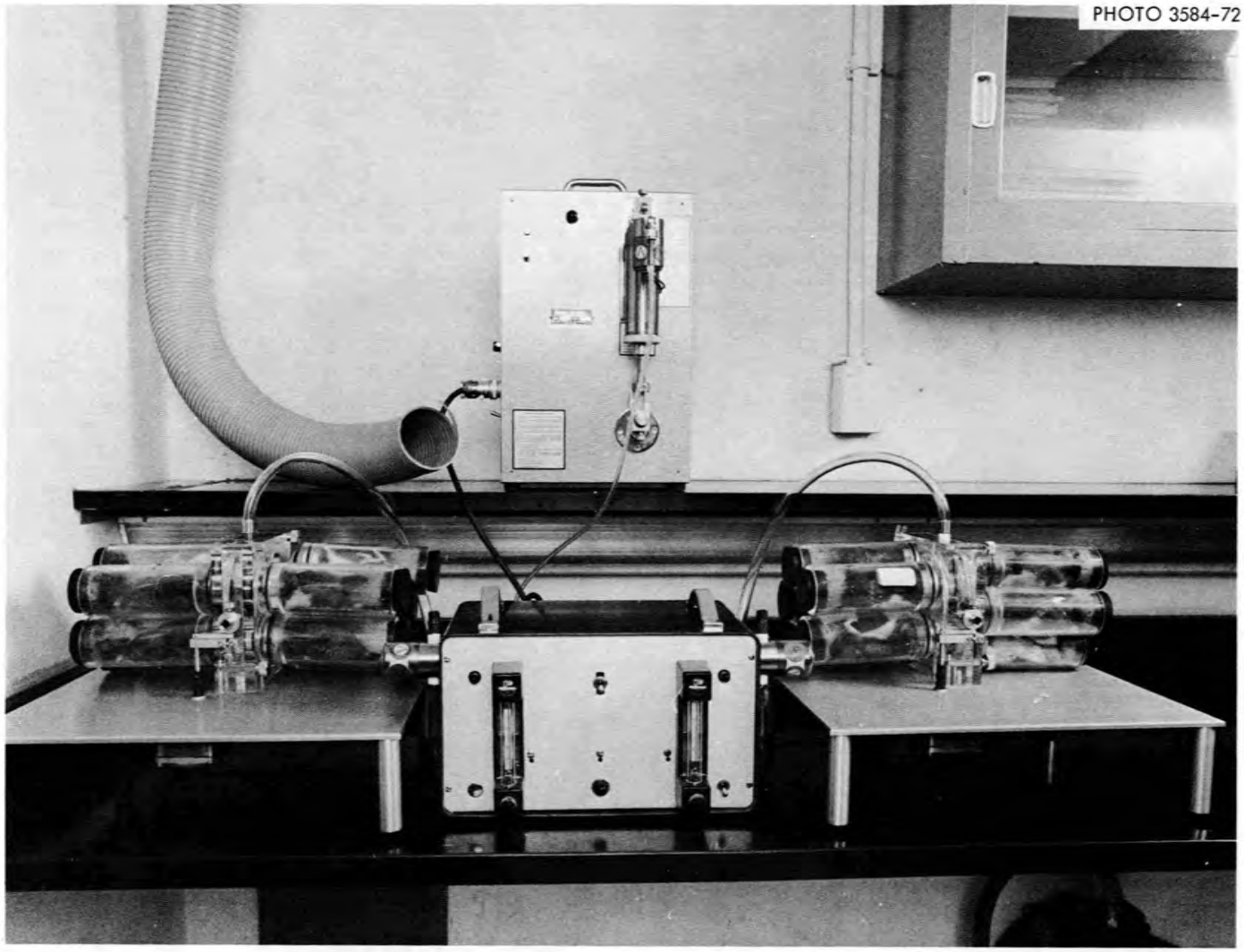
Figure 5-4 Profiles of Gas Phase - ADL Machine Position, Analytically Generated Smoke and Simulated Dog Smoking (sec. 5.4c).

FIGURES

Section 7:00

Figure 7-1 Basic Inhalation Exposure System Modified to Expose Twenty Animals Using a Single Pump.

Figure 7-2 a & b Slant-Faced Animal Containment Tube and Exposure Chamber With Modified Side Plates.



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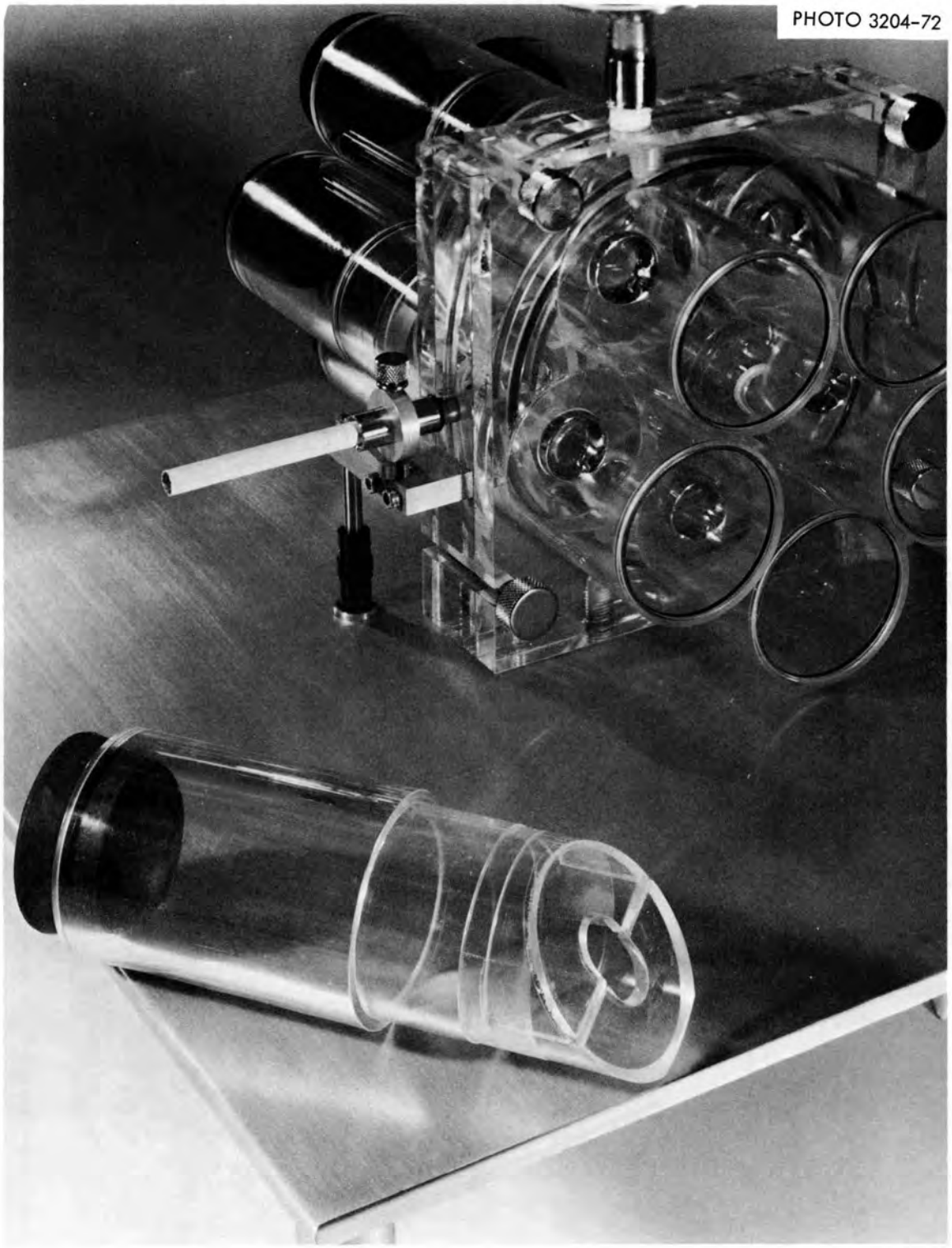


Figure 7-2a Slant-Faced Animal Containment Tube and Exposure Chamber With Modified Side Plates.

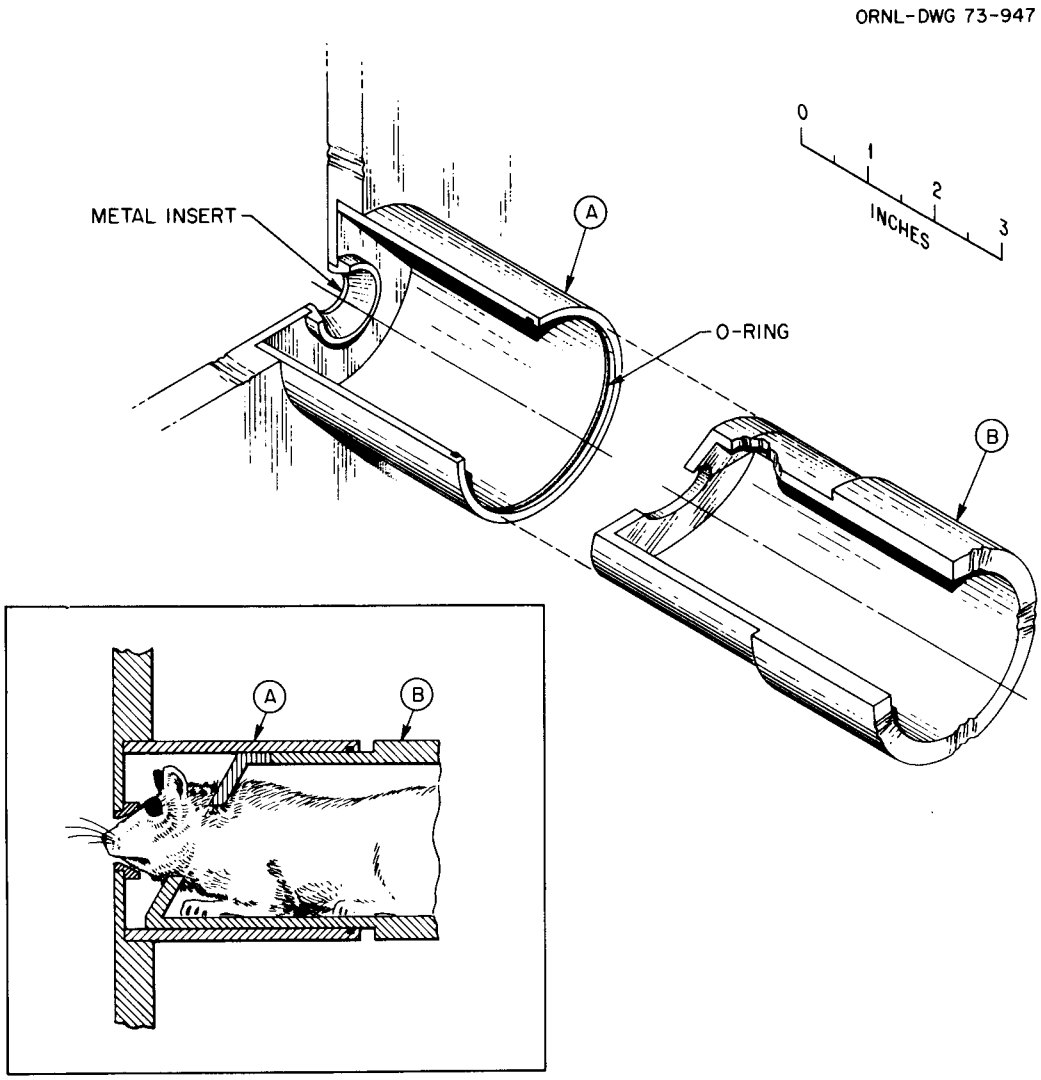


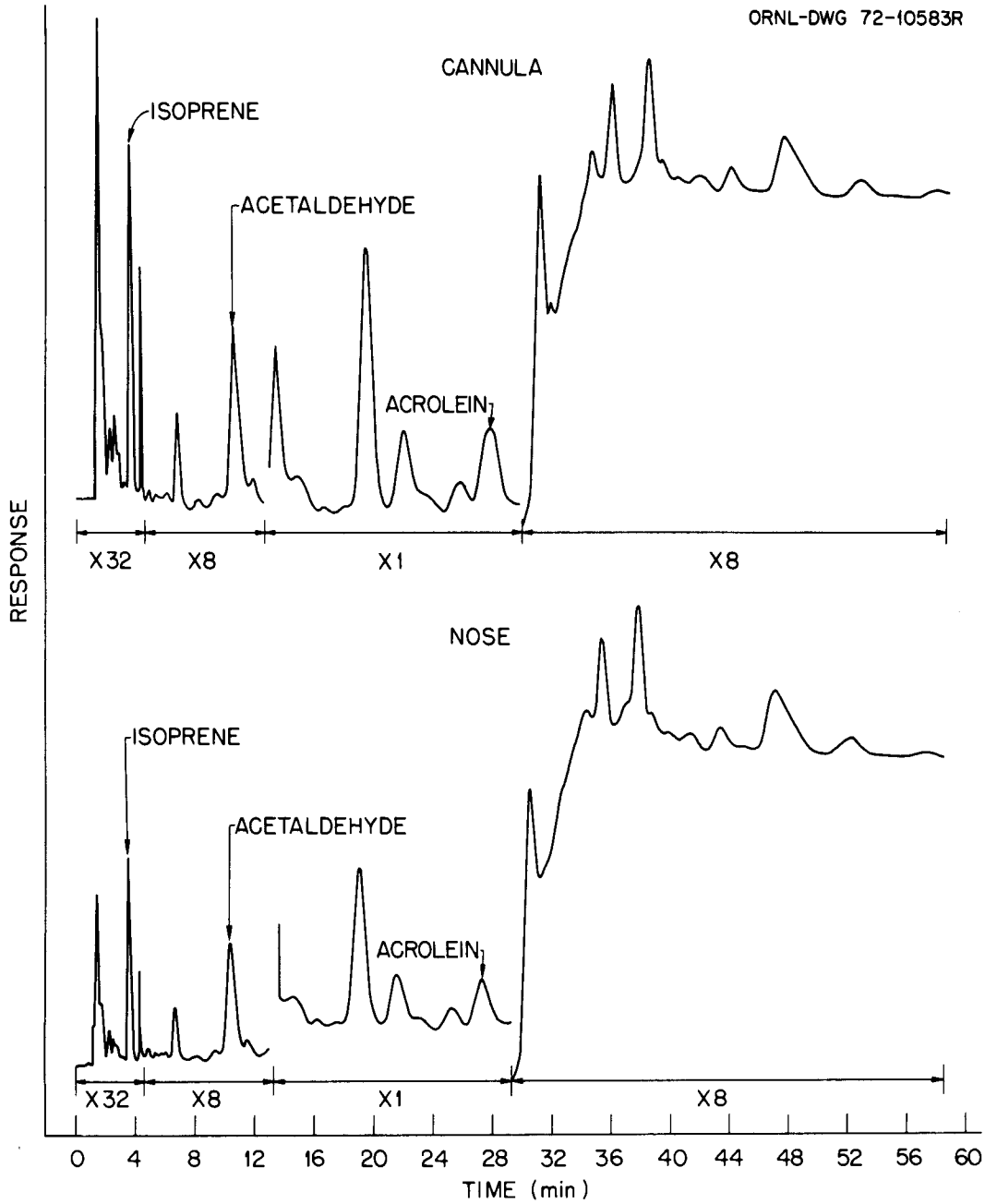
Figure 7-2b Slant-Faced Animal Containment Tube and Exposure Chamber with Modified Side Plates.

FIGURES

Section 8:00

Figure 8-1 Extraction of Gas Phase Components by Upper Respiratory Tract.

Figure 8-2 Tracheostomy Designs for Small Rodents.



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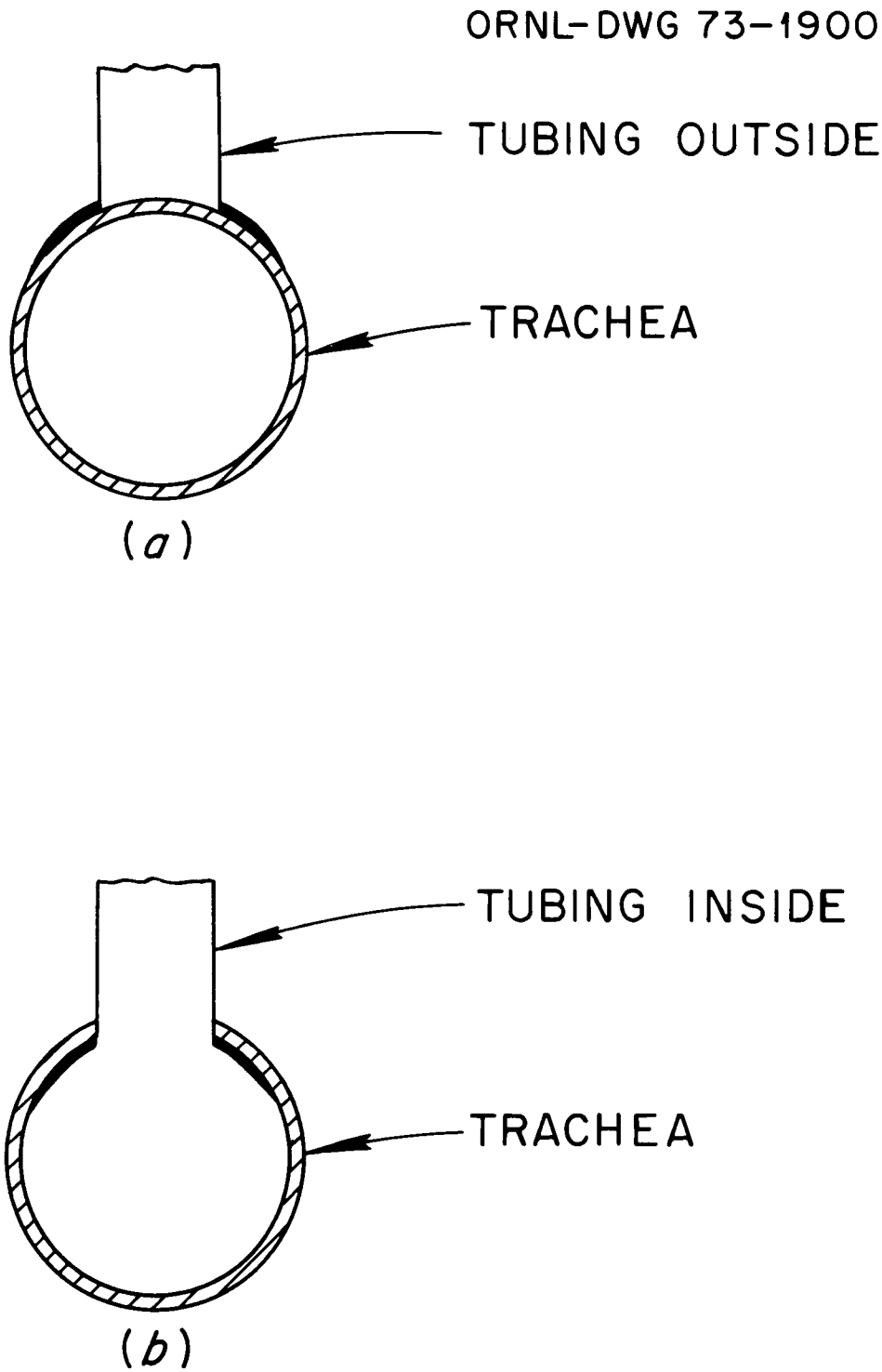

Figure 8-2 Tracheostomy Designs for Small Rodents.

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