ANNUAL PROGRESS SUMMARY

and

TECHNICAL PROPOSAL

to

The Council for Tobacco Research-USA, Inc.

on

CHARACTERIZATION OF ANIMAL INHALATION EXPOSURE DEVICES

and

MICE DOSIMETRY STUDIES ON THE WALTON HORIZONTAL SMOKING MACHINE

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February 6, 1976

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ANNUAL PROGRESS SUMMARY

CHARACTERIZATION OF ANIMAL INHALATION EXPOSURE DEVICES

I. INTRODUCTION

This report summarizes progress on the project to characterize animal exposure devices for the period January, 1975 to January, 1976. The primary objectives of the project are to establish methodology for evaluating inhalation exposure devices, to apply this methodology to devices of interest to CTR, and to formulate recommendations for elimination of discovered shortcomings in the devices.

II. CURRENT STATUS

In this report period, emphasis and priority have been placed on expediting development of the Process and Instruments Smoke Exposure Machine (SEM). A prototype model (SEM I) has received a thorough evaluation of operational characteristics to discover possible shortcoming. Recommendations for improvements in the SEM were made and are incorporated into a new model of the smoking machine (SEM II) which has been built and is presently undergoing tests by the manufacturer. Evaluation and study of the new model (SEM II) will be performed by us in the near future.

Prototype models of mice containment/exposure units designed by Process and Instruments Corp. (P&I) have been evaluated. In cooperation with P&I, we have made design changes in the containment/exposure units to decrease animal stress, prevent animal injury, and provide for more relevant inhalation exposures. Chemical studies were made with the prototype unit to establish the number of mice which could be simultaneously exposed with minimal animal effects on smoke composition and carbon dioxide build-up. A final model of the mice containment/exposure unit is presently being constructed for testing by us and installation at Microbiological Associates.

Study and evaluation of the Walton Horizontal smoking machine were extensive during previous contract periods. Additional characterization/evaluation of this smoking machine have been made during this contract period using newly developed methodology. An air humidification assessory was designed and tested

to provide air of 60% relative humidity to the animals. Puff pressure profiles were measured and compared with a small sampling of human puff profiles. New analytical methods for analysis of specific smoke components in smoke inhalation exposure atmospheres are presently being used to investigate smoke composition in the Walton exposure chamber and the possible effect of animals on smoke composition. It is anticipated that most proposed studies on the Walton smoking machine will be completed by the end of this contract period (July, 1976). Oral presentations of studies on the Walton have been made, and papers for open literature publication are being prepared.

Work on the Lorillard LACS II exposure system has been minimal during this contract period due to failure of electronic components in the system. Major problems remain with the system in the design of the smoke distribution valve and construction of electronic components of the system. Cost estimates were obtained for upgrading the electronic components of the system for possible future use by CTR. At our recommendation and with approval of CTR, efforts on the LACS II were redirected to expedite development of the SEM exposure system.

III. MAJOR PROJECT ACTIVITIES FOR THE PERIOD JANUARY, 1975 - JANUARY, 1976

A. Characterization and Design Improvements in the P&I SEM Exposure System

1. It has been found that significant differences in puff volumes are possible for cigarettes smoked on the SEM. Experiments with unlit cigarettes show that the puff volume depends on the airflow resistance of the cigarette, which can be highly variable from cigarette-to-cigarette and puff-to-puff. (The SEM is a constant pressure smoking machine whereas most other smoking machines are constant volume puffing which assures puff volume consistency.) Direct measurement of puff volumes of individual lit cigarettes on the SEM is not yet possible, but calculations show that puff volume variabilities as high as ±10 ml may be obtained. Methodology for direct measurement of the puff volumes on individual, lit cigarettes is under development to further investigate this potentially serious shortcoming of the new model (SEM II) exposure system.

2. The relative humidity of air in the dome area of the SEM system was found to decrease to below 10% during exposures because dry, pressurized air was used for the dome air supply. Because of the possible effects of low humidity on the burning rate of cigarettes, the chemical composition of the smoke, and deposition of smoke particulates in the animals, several methods for humidifying the dome air supply were investigated. A humidification system which involved forcing the air supply through a water saturator proved satisfactory and is included in the final model (SEM II) of the system.

3. Gas chromatographic analysis of smoke issuing from the SEM revealed that high boiling smoke constituents in the gas phase of the smoke were almost completely absent. Further investigation showed that the smoke constituents were being adsorbed by the tygon smoke transfer lines (Figure 1). Other tubing materials were investigated as substitutive transfer lines. Teflon, acrylic, aluminum, and copper were found to be suitable tubing materials (Table 1) insofar as losses of gas phase constituent is concerned. Previous studies have shown that teflon tubes retain very small amounts of smoke particulates. All smoke transfer lines in the SEM have been converted to teflon or teflon lined rubber tubing.

4. A prototype animal containment unit built for use with the SEM was evaluated to establish the maximum number of animals which could be exposed without appreciably affecting the concentration and composition of smoke offered with the system. Carbon monoxide was used as a marker compound to ascertain animal depletion of highly adsorbed smoke compounds from the smoke stream. It was found that with a full load of mice (20,C57) on the containment unit, the carbon monoxide concentration in the smoke exiting from the unit was 19% less than the concentration entering the unit with one-tenth of the smoke output from the exposure system passing through the containment unit. Carbon dioxide analysis on smoke exiting from the containment unit showed that the ani-

mal add significant amounts of carbon dioxide to the smoke. The carbon dioxide level at the exit under the above conditions was 3-4% (volume). Based on the measured depletion of carbon monoxide and build-up of carbon dioxide, recommendations were made on the maximum number of mice that can be exposed on the SEM simultaneously under different exposure conditions. (Topical Report CTR/ORNL #1 - 9-24-75).

5. Prototype animal containment units were evaluated and recommendations were made for improvements to decrease animal stress, to prevent animal deaths, and to increase operational ease (Topical Report CTR/ORNL #3 - 11-3-75). Improved containment units are presently being made for utilization at Microbiological Associates.

6. Pressure drops on prototype animal containment units were measured to aid in the design of the animal containment system for the SEM (Topical Report CTR/ORNL #2 - 9-28-75).

7. Design changes have been recommended to the SEM manufacturer to eliminate leakage problems at the drum teflon slider block, to prevent plugging of smoke transfer tubes and lines, and to provide provisions for sampling of smoke for quality control and machine maintenance purposes.

8. The mechanical and electronic components of the SEM have been found to be reliable and relatively trouble-free. The system is easily maintained by minimally trained operators. Puff volume calibration is the only operation requiring trained and knowledgeable personnel. (A new puff volume calibration procedure is currently being developed by the manufacturer).

B. Characterization and Design Improvements in the Walton Horizontal Smoking Machine

1. New methodology for measurement for smoke particle size distribution in inhalation exposure systems was applied to the Walton smoking machine. Figure 2 shows typical data on the particle size distribution of smoke sampled from the Walton exposure

chamber at 1, 15 and 30 seconds after smoke enters the chamber. The mean particle diameter is seen to increase by a factor of four during a 30 second exposure period with little change in the shape of the distribution. The work suggests that possible changes in the deposition site in the animal's respiratory system are possible due to aging of the smoke in the exposure chamber. Smoke deposition studies using carbon-14 tracers should be carried out to determine if smoke aging appreciably affects the deposition site of the smoke particulates in animals.

2. A newly developed smoke particulate sensor for monitoring smoke particulate concentration in inhalation exposure systems was used to establish the time and spatial distribution of smoke particulates in the Walton exposure chamber. Figure 3 shows the response of the device at one exposure position in the Walton chamber for eight puffs on a Kentucky Reference 1A1 cigarette. In other experiments, differential measurements made with two smoke particulate sensors showed that smoke particulate are uniformly (within ±2%) distributed in the exposure chamber so that all animals on the system are exposed to smoke of identical concentration. Other studies on the loss of smoke particulates to chamber walls, effect of stirring, and animals effects on smoke concentration are presently being made. (Topical Report CTR/ORNL #4 - 1-23-76 describes in detail the results of completed work with the smoke particulate sensor).

3. Puffing profiles (pressure differential across cigarette during puffing) were obtained for the Walton smoking machine and compared with similar profiles from a small group of human profiles. Figure 4 shows a typical profile taken on the Walton machine on successive puffs from a 1R1 cigarette. For all puffs, there is an initial rapid rise in the pressure differential and a pressure spike at initiation of puffing. The pressure spike is due to the forward motion of the puffing dome and compression of the "O" rings as the puffing dome seals on the cigarette holder assembly. The pressure profile during each puff is very erratic and differs

in appearance from one puff to the next. The erratic and irreprodicible nature of the profiles is thought to be due to non-uniform burning of the cigarette and is not caused by malfunction of the smoking machine. Comparison of pressure profiles obtained on the Walton with human profiles show some differences. Human profiles are, in general, round or bell shaped whereas the Walton profile is square-wave shaped. It is not thought that this difference in profiles is significant because of the high variability of profiles for human smokers.

4. Studies are underway to establish the concentration of and effect of animals on selected smoke components and respiratory gases in the exposure chamber of the Walton smoking machine. The following components are being determined: methane, hydrogen, carbon monoxide, carbon dioxide, neophytadiene, nicotine, and oxygen. Figure 5 shows the effect of mice on the concentration of oxygen in the exposure chamber during a 30 second exposure on the Walton. It is observed that the mice cause a decrease in the oxygen concentration during the exposure. An interesting observation with this data is that the oxygen concentration decreases at a slower rate when smoke is offered compared with the rate in a simulated exposure where no smoke is present. It is apparent that, at least, in the early part of the exposure period, animal respiration is greatly curtailed; the mice sense the presence of the smoke and volunteerly reduce their breathing rate significantly. These studies were performed with unadapted mice; additional studies with fully adapted mice are planned as well as work with other smoke components and carbon dioxide.

5. With the Walton exposure system it is convenient to use pressurized air for puffing and purging of smoke from the exposure chamber. Air must be supplied at a pressure of 25 psi for satisfactory machine operation. Using high pressure air, we found that the relative humidity in the exposure chamber decreased to less than 20% during exposures. An air humidification accessory was designed and installed on the Walton to prevent this undesirable drop in

humidity. With the accessory, the supply air is saturated with water at a controlled pressure and then expanded to atmospheric pressure in the exposure chamber. By adjustment of the air pressure in the water saturator, the relative humidity of the supply air entering the exposure chamber can be adjusted to relative humidities of 40-70 percent. Figure 6 shows a schematic diagram of the humidification accessory.

C. New Methodology for Characterization of Inhalation Exposure Systems

1. We have investigated, further developed, and adapted a technique described by W. L. Carter and I. Hasigawa (28th Tobacco Chemists' Research Conference, October 28-29, 1974, Raleigh, N. C.) in order to measure the particle size distribution of smoke particulates in animal inhalation systems. With this technique, tobacco smoke particles are fixed by reaction with 2-methylcyano acrylates (MCA), collected by filtration, and examined by scanning electron microscopy. Figure 7 is a electron micrograph of smoke particles fixed by this technique. We have validated this new technique by comparison of particle size distributions with distribution obtained previously using acceptable techniques and by ion-etching experiments. Improvements in the MCA technique have resulted from our efforts to apply the technique to smoke in inhalation system. Application of the technique to smoke in the Walton exposure chamber have been made (see section B-1) and additional studies are planned for the SEM II exposure system.

2. A light scattering device has been designed and built for continuous monitoring of tobacco smoke particulates in inhalation exposure systems. The device is useful for studies aimed at establishing the uniformity of smoke in exposure systems, the age of the smoke when it reaches the exposure site, puff time, and other exposure conditions. The device has been used to study the spatial and time distribution of smoke in the Walton exposure chamber (section B-2). Tests made with the device show that the response is linearly related to smoke particulate concentration and that

the response is independent of cigarette type and apparently depends only on the particulate matter concentration in the exposure chamber (Figure 8). There are numerous applications of this device to characterize the Walton and SEM exposure systems. Current plans call for utilization of the device as a smoke monitor in the SEM II exposure system at Microbiological Associates to prevent accidental deaths of animals due to exposure to toxic levels of smoke.

3. A new method for sampling smoke from inhalation exposure systems has been developed. Previous methods used by us were designed for smoke sampling from static (Walton) exposure system and are not applicable to sampling of continuous streams of smoke as generated by the SEM and LACS II smoking machines. In the new method, smoke is sampled by a motor-driven syringe which removes smoke at a selected, constant rate (0.1-40 ml/min). Initiation and termination of sampling is controlled by two timers which supply power to the syringe drive. The two timers permit sampling for a selected interval during any part of the exposure cycle. The timers can be by-passed to provide continuous sampling over the entire exposure cycle. Smoke is withdrawn from the exposure system through a micro Cambridge filter pad which retains smoke particulates, and the gas phase is collected in the glass syringe. Analysis are then made on either or both smoke fractions to determine constituents of interest. This sampling method is being used to sample smoke from the Walton and SEM exposure system to establish smoke concentration and composition (section B-4).

IV. PRESENTATION OF RESULTS

A. Oral Presentations

1. "Chemical Indicators and Methods for Determination of Tobacco Smoke Concentration in Inhalation Exposure Systems", J. R. Stokely, J. H. Moneyhun, and M. R. Guerin, presented at the 29th Tobacco Chemists' Research Conference, College Park, Maryland, October 8-10, 1975.

2. "Depletion of Constituents in Contained Smoke Aerosols for Inhalation Exposure Dosimetry", J. E. Caton, J. R. Stokely, and M. R. Guerin, presented at the 29th Tobacco Chemists' Research Conference, College Park, Maryland, October 8-10, 1975.

3. "Organic Gas Phase Composition of Contained Smoke Aerosols Used for Inhalation Testing", C. E. Higgins, J. R. Stokely, and M. R. Guerin, presented at the 29th Tobacco Chemists' Research Conference, College Park, Maryland, October 8-10, 1975.

4. "Electron Microscope Measurement of Methyl Cyanoacrylate Fixed Tobacco Smoke Particles", R. W. Holmberg, J. R. Stokely, and M. R. Guerin, presented at the 29th Tobacco Chemists' Research Conference, College Park, Maryland, October 8-10, 1975.

5. "Chemical and Physical Properties of Smoke in Inhalation Exposure System", J. R. Stokely, presented at NCI Contractors' Conference, Atlantic City, N. J., July 17-18, 1975.

B. Reports

1. "Initial Evaluation of a Prototype Animal Containment Unit for the P&I SEM", J. H. Moneyhun and J. R. Stokely, CTR/ORNL Topical Report #1 - 9-24-75.

2. "Pressure Measurements in a Prototype Animal Containment Unit for the P&I SEM", J. H. Moneyhun and J. R. Stokely, CTR/ORNL Topical Report #2 - 9-28-75.

3. "Observations and Suggestions on the Prototype Animal Containment Unit for the P&I SEM", J. H. Moneyhun and J. R. Stokely, CTR/ORNL Topical Report #3 - 11-3-75.

4. "Monitoring Smoke Concentration in the Walton Exposure Chamber Using a Light-Scattering Sensor", C. E. Higgins, T. M. Gayle, and J. R. Stokely, CTR/ORNL Topical Report #4 - 1-23-76.

TABLE I
LOSSES OF GAS PHASE COMPONENTS FROM SMOKE PASSING
THROUGH TUBING OF VARIOUS MATERIALS

Tubing length: 10 ft., smoke generated from 1A1 cigarettes by P&I SEM Smoking machine, flow rate through tubing: 10.5 l/minute - 10% smoke and 1.05 l/minute - 100% smoke. Smoke sampled at entrance and exit of tubing by pulse sampling with gas sampling valve and transferred to gas chromatograph. High resolution chromatogram obtained under standard conditions.

Tubing Material	Tubing I.D., in.	Smoke Conc, %	Percent Loss			
			Benzene	Toluene	m-Xylene	d-Limonene
Aluminum	3/16	100	<5	<5	<5	<5
Acrylic*	1/4	100	5	<5	<5	<5
Copper	3/16	100	<5	<5	8	<5
Teflon	3/16	100	<5	<5	<5	5
Teflon	3/16	10	<5	<5	<5	<5
Stainless Steel	3/8	100	<5	<5	27	28
Polyethylene	3/16	100	<5	<5	21	32
Polyethylene	3/16	10	<5	<5	<5	<5
Latex Rubber	1/4	100	18	45	55	74
Latex Rubber	1/4	10	14	<5	<5	19
Tygon	3/8	100	18	45	55	74
Tygon	3/8	10	<5	<5	<5	21
Tygon	3/16	10	<5	<5	<5	20
Silicone Rubber	1/4	100	51	94	100	100

* 6 feet in length

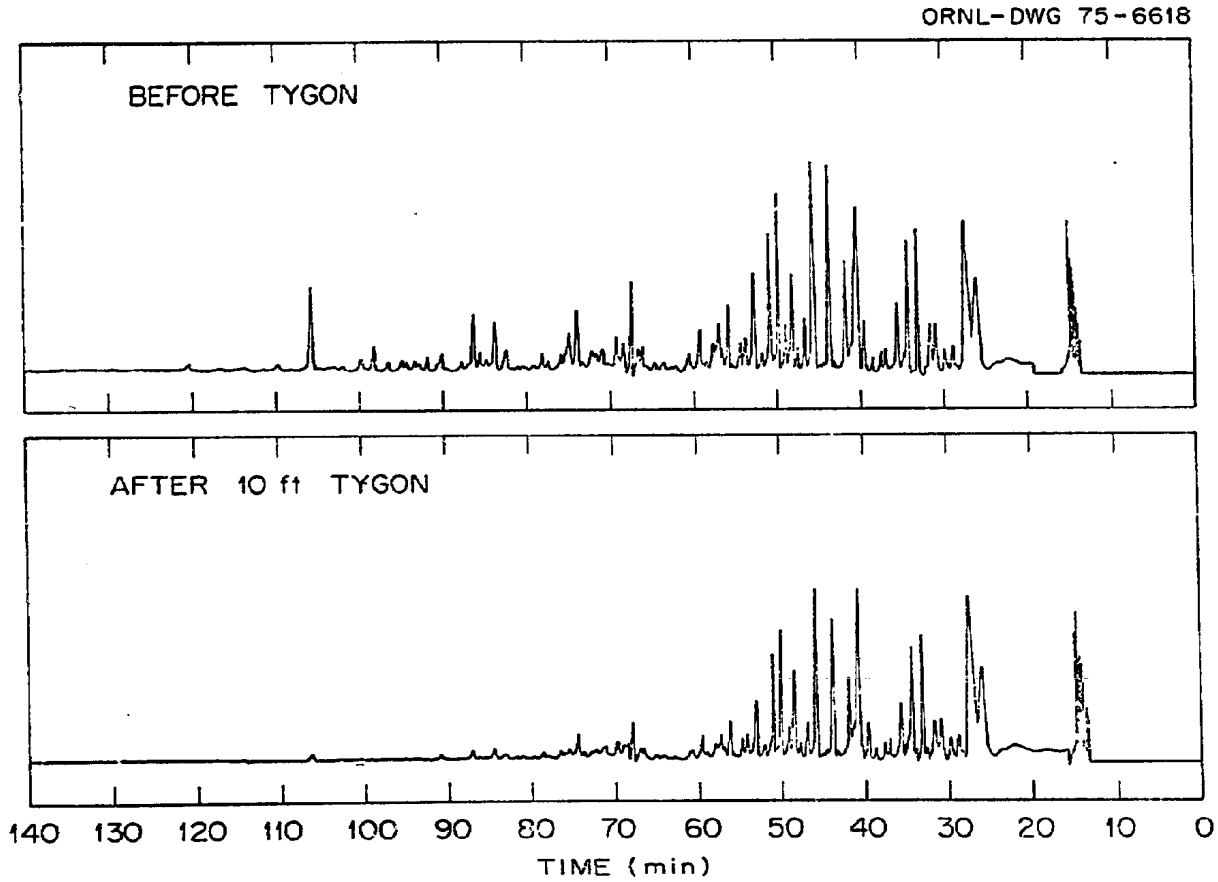

FIGURE 1. Gas Chromatographic Profiles of Organic Gas Phase Constituents in Smoke Sampled Before and After Passage Through Ten Feet of Tygon Tubing.

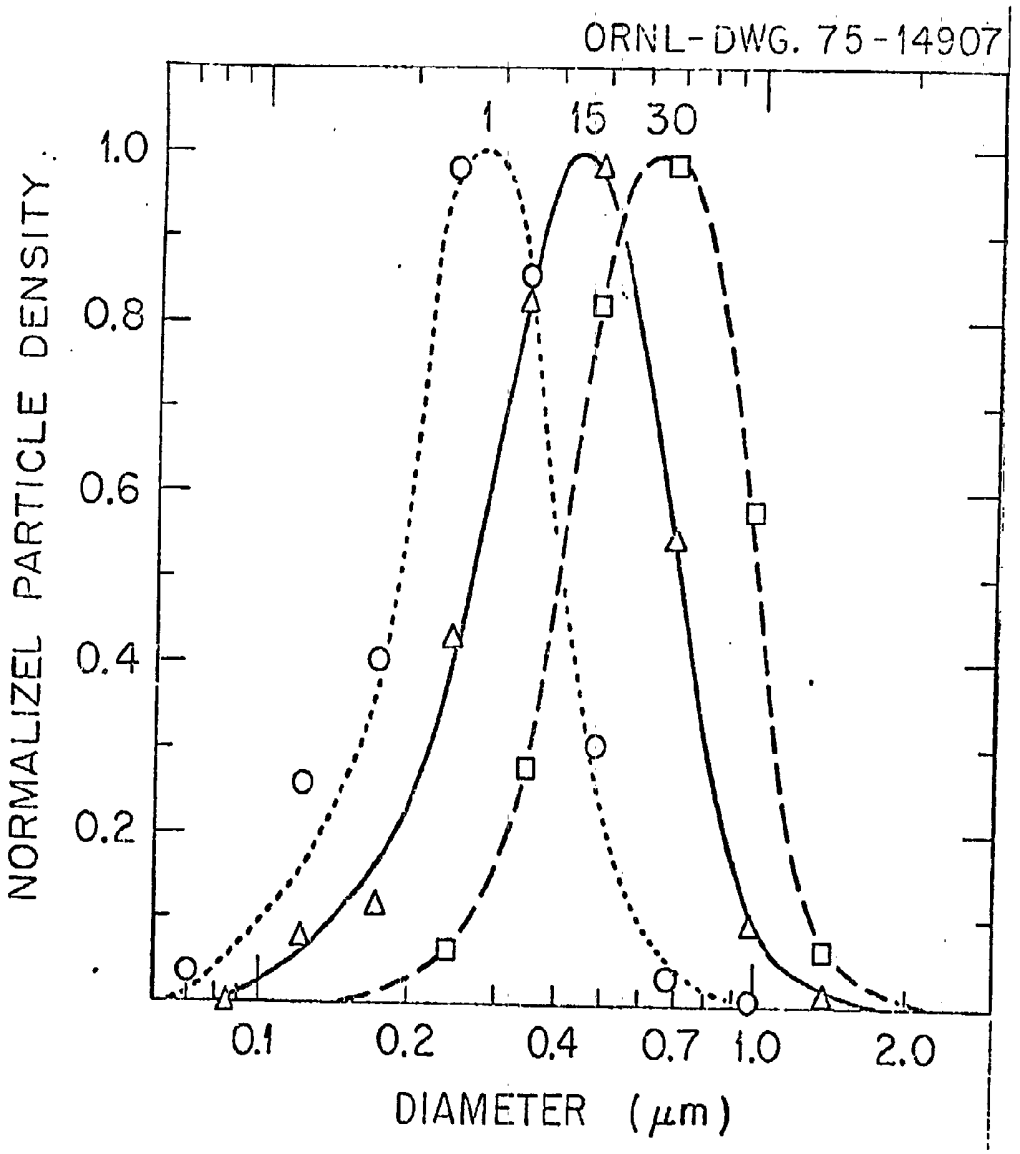

FIGURE 2. Particle Size Distribution of Smoke Sampled from Walton Exposure Chamber at Indicated Times (Seconds) after Cigarette was Puffed.

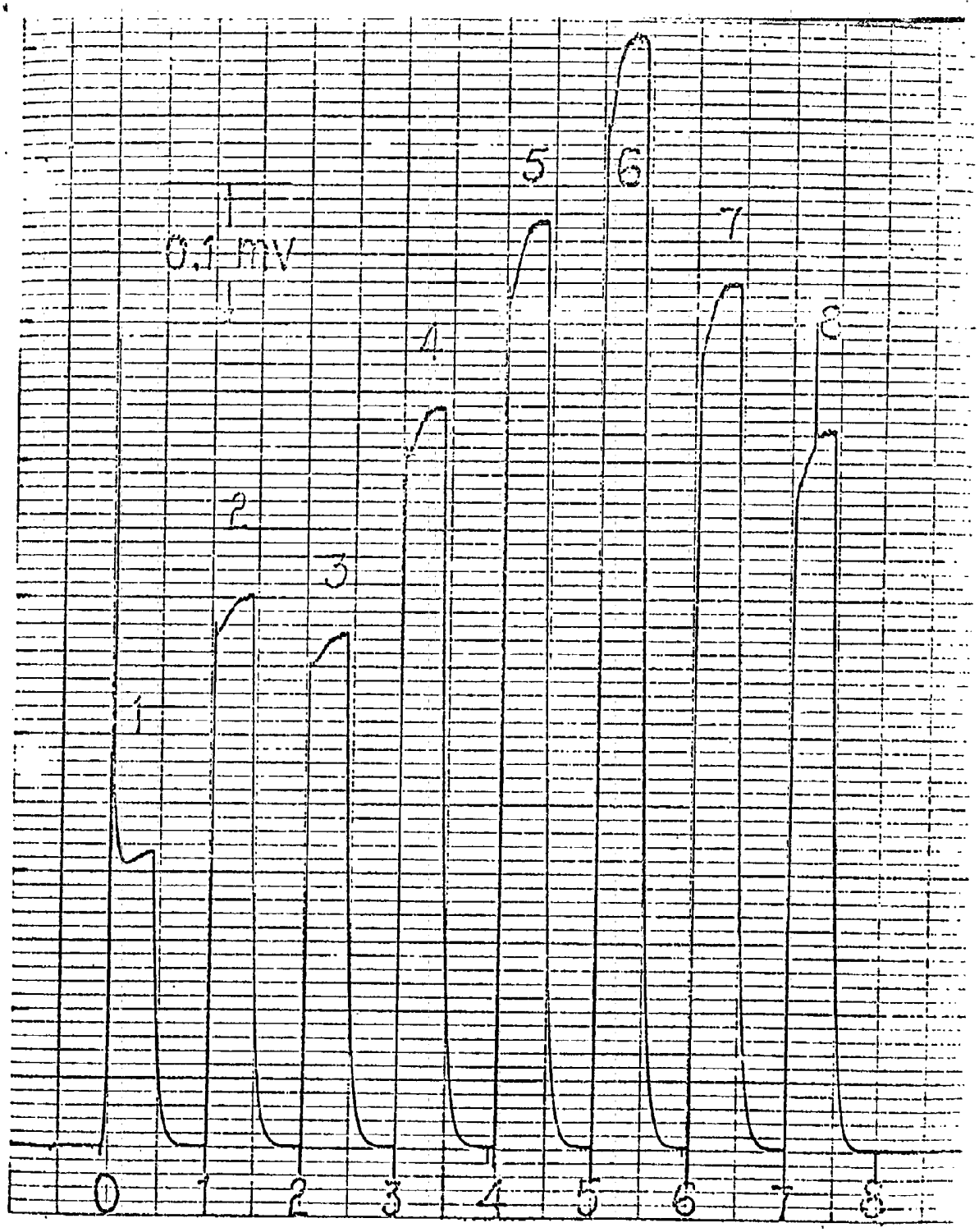

FIGURE 3. Light Sensor Response to 1Al Smoke in the Walton Chamber on a Puff by Puff Basis.

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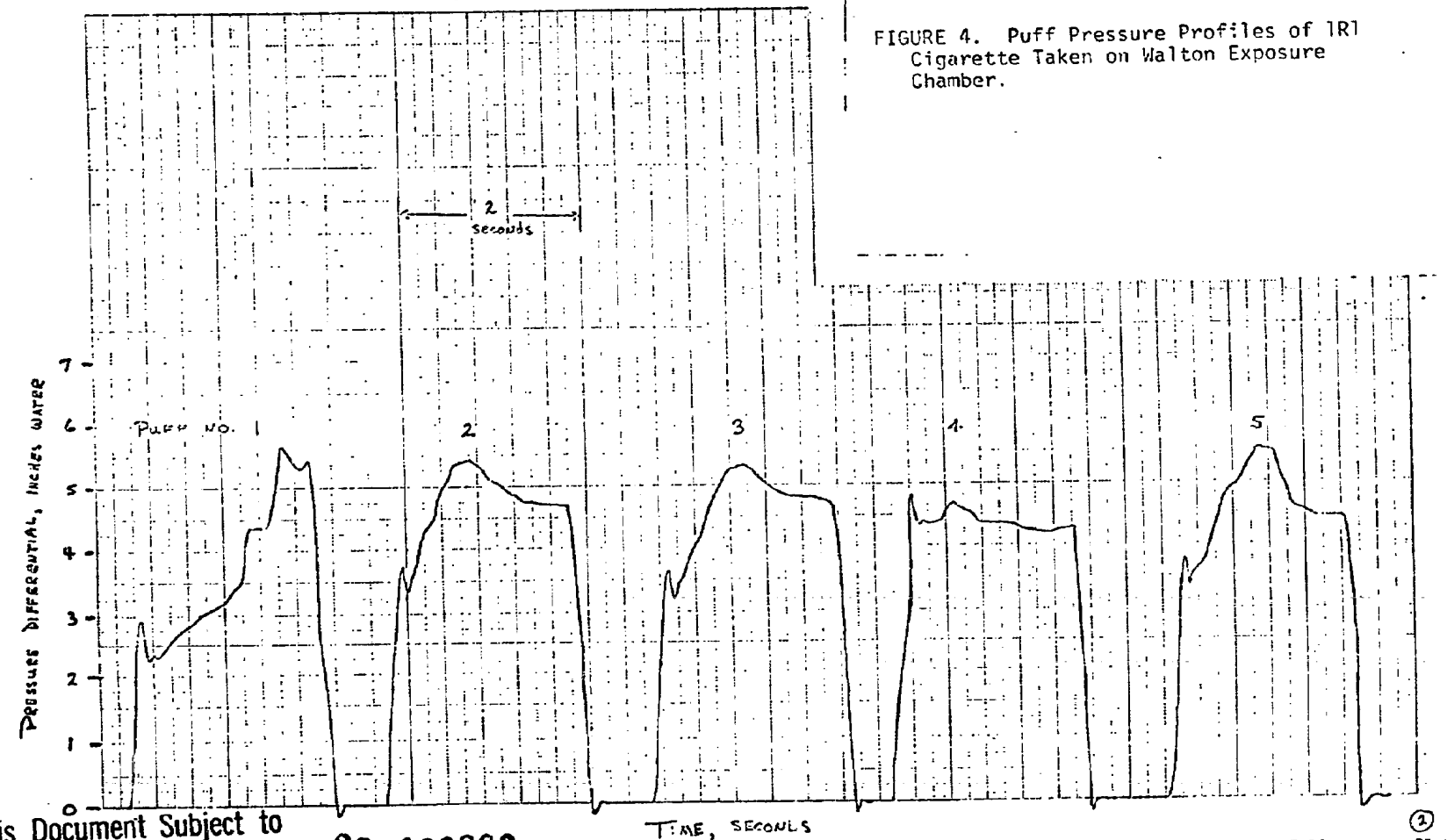


FIGURE 4. Puff Pressure Profiles of 1R1 Cigarette Taken on Walton Exposure Chamber.

FIGURE 5. Effect of Mice on Concentration of Oxygen in Walton Exposure Chamber With and Without Smoke in Chamber.

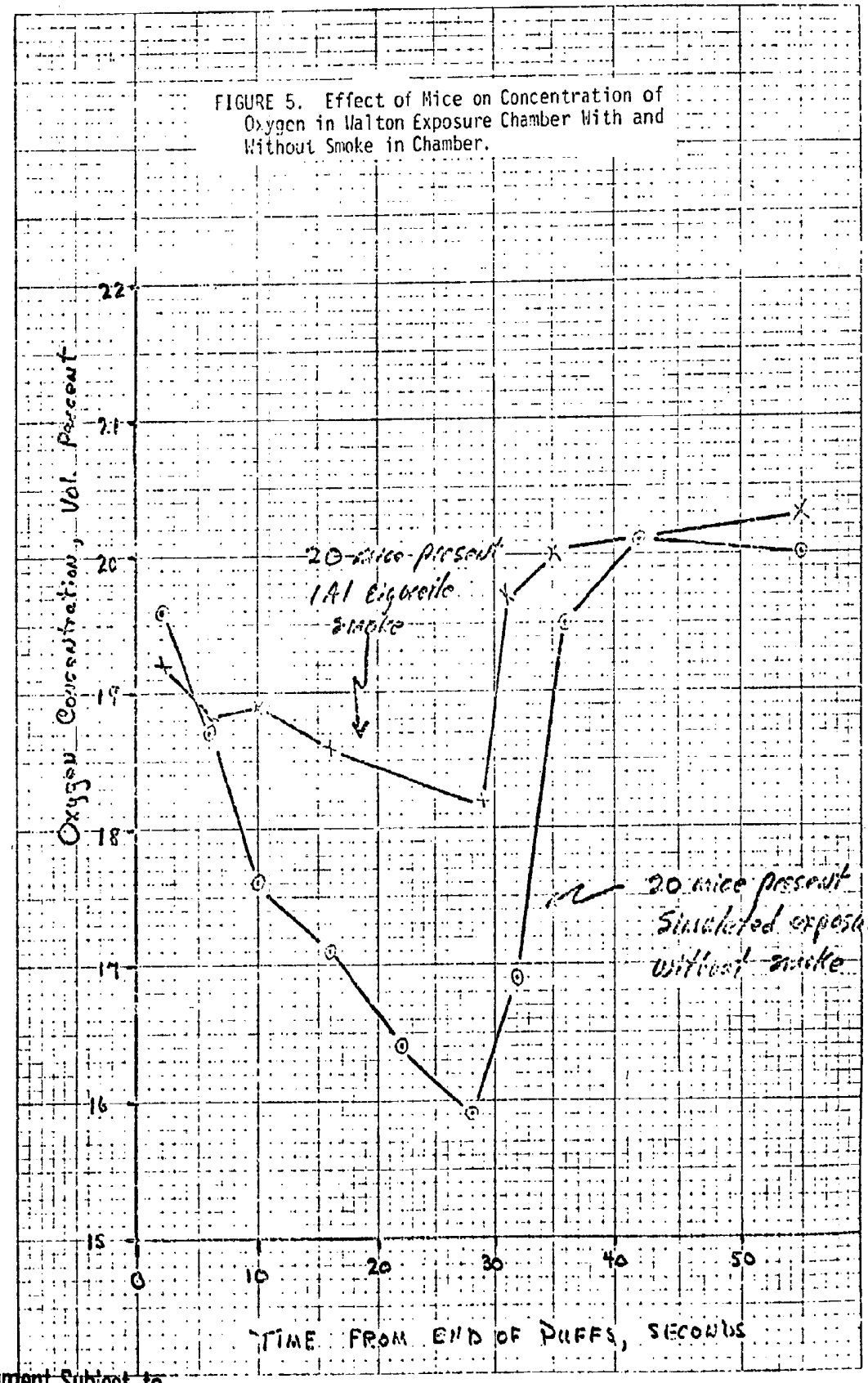
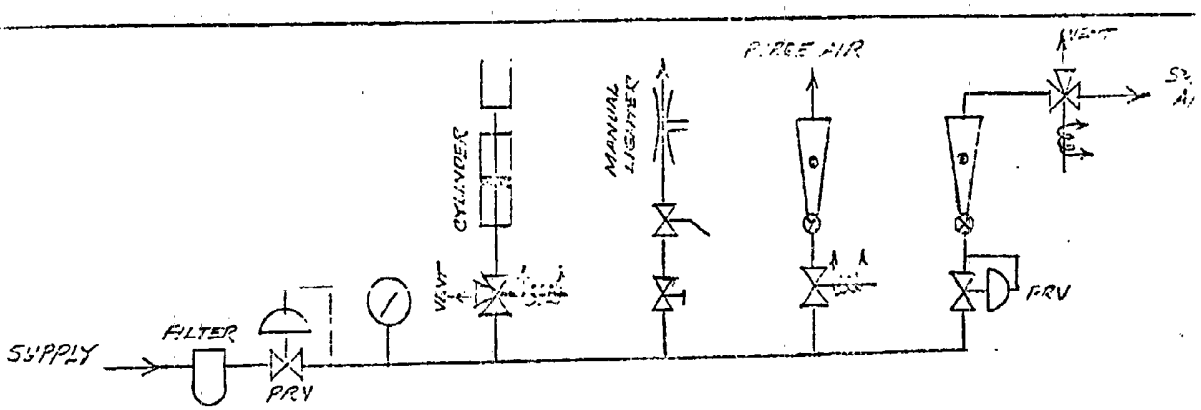
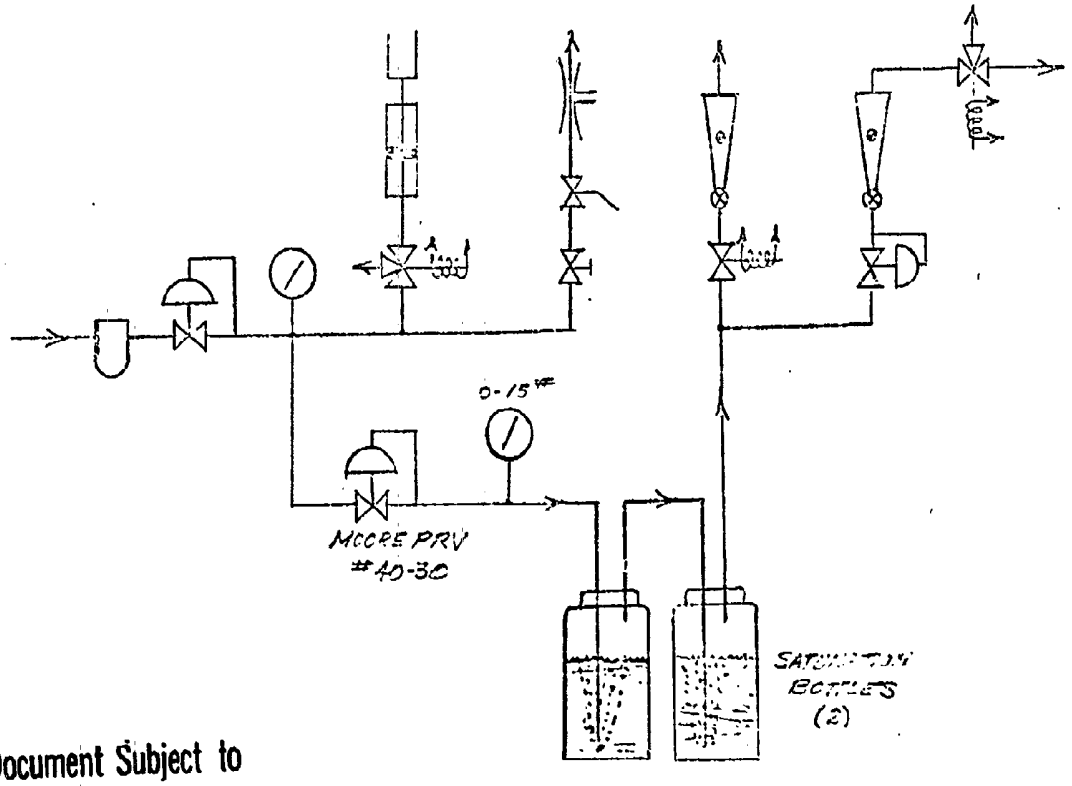


FIGURE 6. Original and Revised Flow Schematic to Provide Humidification of Air Supply on Walton Horizontal Smoking Machine.



ORIGINAL PIPING



REVISED PIPING

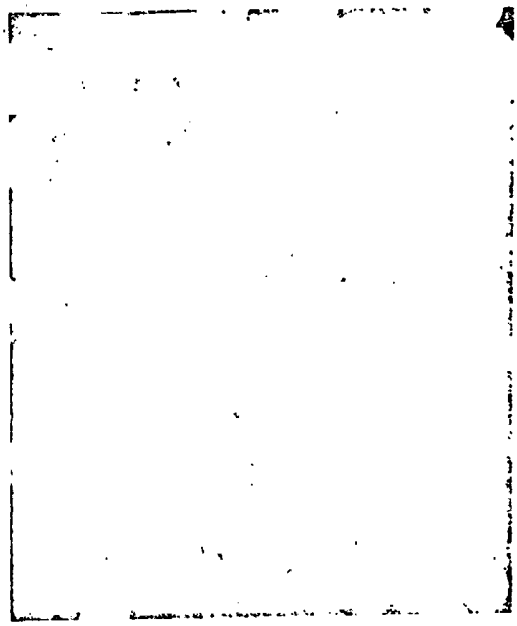
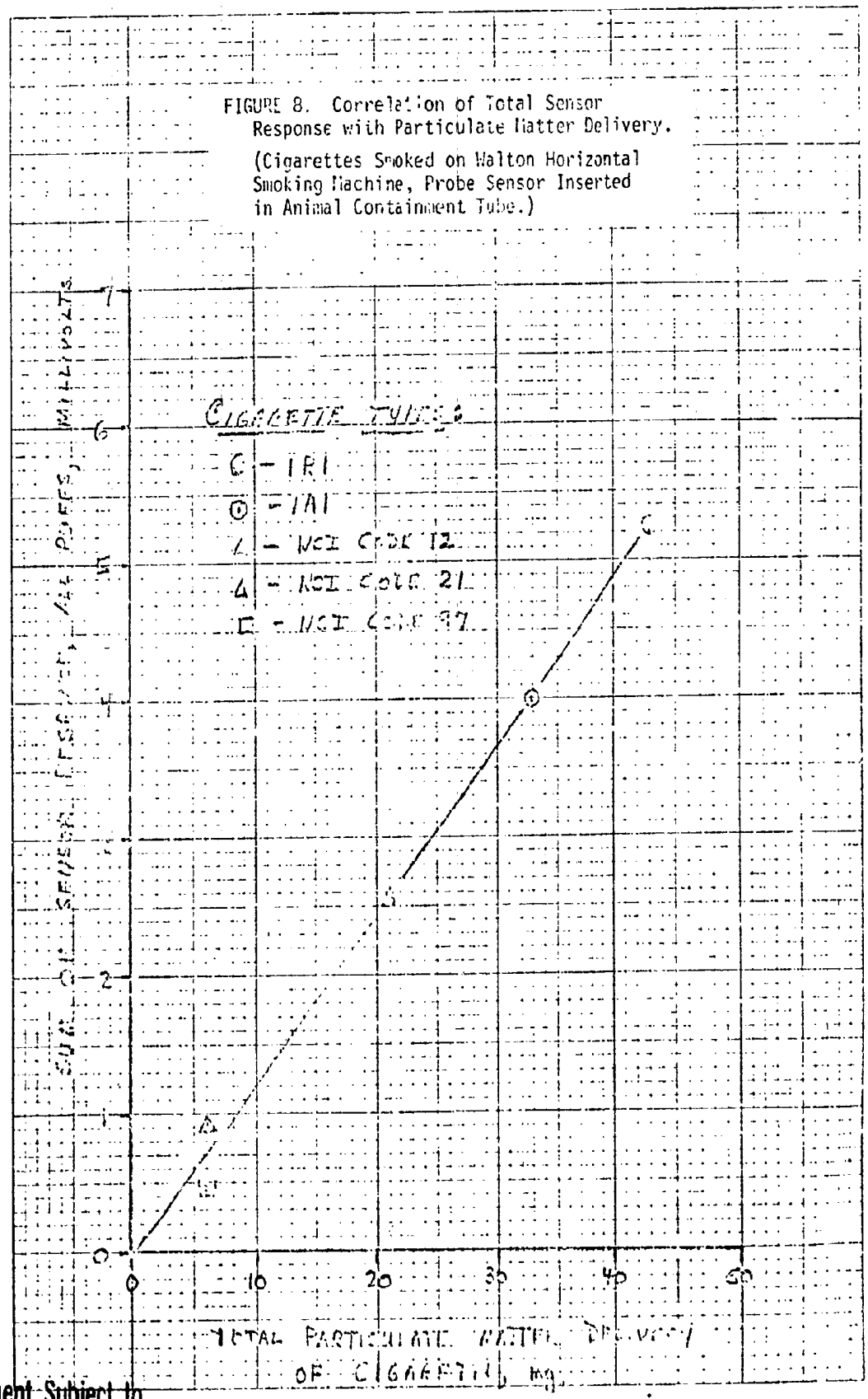


FIGURE 7. Scanning Electron Micrograph of Cigarette Tobacco Smoke Encapsulated with MCA and Collected on 0.1 μm Nucleopore Filter. Magnification: 20,000 X.

FIGURE 8. Correlation of Total Sensor Response with Particulate Matter Delivery.

(Cigarettes Smoked on Walton Horizontal Smoking Machine, Probe Sensor Inserted in Animal Containment Tube.)



PROPOSAL FOR NEW STUDIES - CHARACTERIZATION OF ANIMAL INHALATION EXPOSURE DEVICES
(August 1, 1976-1977)

I. INTRODUCTION

The purposes of this project are to (1) collaborate in the development of advanced tobacco smoke inhalation exposure systems and methods, and (2) to characterize the exposures provided by exposure devices of interest to CTR. Carefully defined exposure systems are critical to the proper design and final interpretation of biological studies requiring inhalation dosing or dosing with whole smoke using other biological models. Continuing effort in this area is required to finalize selected studies for publication and to provide characterization and documentation of exposures provided by the Process and Instruments SEM II exposure system.

II. PROPOSED PROJECT ACTIVITIES

Experimental studies will emphasis (a) extension of on-going work on the Walton smoking machine, (b) initiation of studies to characterize exposures provided by the SEM II exposure system, (c) final development and application of advanced methodologies to characterize the Walton and SEM II exposure devices, and (d) initiation of a collaborative effort with Microbiological Associates to provide chemical/instrumentation support for chronic mouse inhalation exposures. Specific projects to be performed under this contract are enumerated below:

1. On-going studies to establish the concentration of and effect of animals on primary smoke constituents in the Walton smoking machine will be expanded to include hydrogen cyanide, formaldehyde and hydrogen sulfide. These three constituents are of interest because they are major smoke constituents and are chemical reactive and physiologically active. Analytical methodology is presently available for determination of these constituents in smoke, and this methodology will be adapted to exposure chamber analysis.

HCN
C-O
H_2S

2. On-going studies to investigate the effect of aging on the particle size distribution of smoke aerosols in the Walton exposure chamber will be extended to include studies on the effect of stirring and mice on the particle size distribution.

3. Operational characterization of the SEM II exposure system will be performed to establish the humidity and temperature of puffing and purge air, consistency of dome pressure, operational reliability and ease, reliability of new puff-volume calibration method, and service and cleaning requirements. The system will be operated for extended periods to establish machine component reliability.

4. In-depth studies will be made to establish the puff volume consistency (from cigarette-to-cigarette) of cigarettes smoked on the SEM II. (Completed studies on the SEM I indicate that there may be large and uncontrollable differences in puff volumes). Apparatus will be developed and used to continuously measure and record puff volumes as cigarettes are smoked on the SEM II. If large differences in puff volumes are encountered, chemical studies will be made to ascertain if the puff volume variability affects the chemical composition of smoke generated by the system and/or means will be devised to reduce the variabilities.

5. Methods for absolute measurement of smoke concentration will be utilized to establish the concentration and delivery of smoke generated by the SEM II under exposure conditions (smoke dilution, smoke flow rate, etc.) identical to those used in chronic inhalation exposures at Microbiological Associates.

6. Studies will be made to determine the chemical composition of smoke generated by the SEM II and to compare this smoke with smoke generated under standard analytical smoking conditions. The following constituents or smoke fractions will be investigated: gas and semi-volatile smoke constituents by high resolution gas chromatographic profiling, major particulate matter components by gas chromatographic profiling, and specific analysis for nicotine, carbon monoxide, carbon dioxide, methane, neophytadiene, hydrogen cyanide, acetaldehyde, isoprene, formaldehyde, and hydrogen sulfide.

7. The particle size distribution of smoke generated by the SEM II will be established and compared with distribution obtained on fresh whole smoke generated under analytical smoking conditions.

2 Experimental studies will be made to determine if the SEM II can be utilized to produce smoke aerosols of artifically large or small particle size distribution for studies of the importance of particle size in dosimetry and impact.

8. As the SEM II is being considered for inhalation exposures at very low smoke concentrations (1%), studies will be made to establish the possible effects of high smoke dilution on the particle size distribution and the particulate matter/gas phase distribution of primary smoke constituents. Existing methodology (MCA encapsulation, high resolution gas chromatographic profiling) will be utilized for these studies.

9. The final model of the SEM II mouse containment/exposure unit will be evaluated with special attention to the following factors: animal stress, smoke leakage, ease of loading and unloading animals, cleaning frequency, prevention of animal deaths and injuries, and possible effect of animals breathing on smoke composition. The containment/exposure unit will be tested with each strain of mouse under consideration for chronic exposure at Microbiological Associates.

10. Techniques will be developed to measure carbon dioxide produced by animals undergoing smoke exposure on the SEM II. This technique will be utilized in dosimetry studies (Part II, this proposal) to determine if carbon dioxide production by the animals can be empirically correlated with the dose of smoke particulates deposited in the respiratory tract. If this method proves valid, then it will be possible to continuously monitor dosimetry in mice in chronic inhalation exposures with the SEM II.

11. A collaborative effort will be initiated with Microbiological Associates in the selection and quality control of cigarettes used in chronic mouse exposure experiments. Cigarettes considered for use in the exposures will be analyzed for smoke constituents deemed to

be important and for which routine analysis methods are available. Quality control procedures will be devised and methodology will be made available to Microbiological Associates to provide quality control of cigarettes used in inhalation studies.

III. REPORTING OF RESULTS

Informal reporting of the status and experimental results derived from this project will be made to the CTR project officer at monthly intervals. CTR/ORNL Topical Reports will be prepared and submitted to CTR when deemed appropriate by the principle investigator or CTR project officer. A comprehensive report of work carried out under this contract will be prepared and submitted to CTR upon completion of experimental studies. Results of experimental studies, if deemed appropriate by in-house review, will be submitted for open literature publication or oral presentation. CTR will be informed before publication or presentation of experimental findings. Selected topics resulting from this working will be included in the Tobacco Smoke Research Program Annual Progress Report published by ORNL.

IV. STAFFING AND BUDGET

Man Yr Allocation	Name and Function
0.50	J. R. Stokely (Ph.D); Principle Investigator, experimental planning and design.
0.75	R. W. Holmberg (Ph.D); Development of particle size distribution methodology and application to exposure systems.
1.00	L. B. Yeatts (Ph.D); Routine smoke composition studies, sampling and analysis for smoke concentration measurements.
1.00	J. H. Moneyhun (B.S.); Operational studies on SEM II, evaluation and design of animal containment unit, chemical analysis development and application
0.75	C. E. Higgins (B.S.); High resolution gas chromatographic analysis, applied studies with continuous smoke concentration monitors, routine chemical analysis.
0.25	T. M. Gayle (B.S.); Mechanical and electronic design and construction.
1.00	D. D. Pair (Tech); Routine chemical analysis, animal care and handling.

Cost Distribution

1. Salary and Overhead (229,000). To include 2.25 man-yrs Ph.D chemist, 2.0 man-yrs B.S. chemist, and 1.00 man-yr non-degreed analyst.

2. Materials and Supplies (16,000). To include normal in-house stores supplies and outside procurement of consumables. Major items include:
   (a) Gas chromatographic supplies (carrier and detector fuel gases, syringes, column packings, tubings, fittings, chart paper, septums, etc.).
   (b) Chemicals (standards, tracers, solvents, eluent, reagents, etc.).
   (c) Smoke generation and collection (filter pads, filter assemblies, cold traps, saran/tedlar bags, cigarette conditioning vessels, etc.).
   (d) Components and raw materials (valves, timers, switches, pumps, piping, instrument components, etc.).

3. Construction and Maintenance (20,000). To include in-house fabrication of items under development or not commercially available and maintenance of existing instrumentation. Major items include:
   (a) Monitoring systems (particulate sensor, chemical sensors).
   (b) Sampling systems (continuous and pulse).
   (c) Smoke generation systems (for comparisons of push/pull puffing, to generate reference data, to study TPM/gas phase distributions, etc.).
   (d) Cigarette loader (to prepare cigarettes doped with specific constituents).
   (e) Particle size (sampling and filtration systems).
   (f) Routine maintenance.

4. Travel (7,000). To include trips to Microbiological Associates, Process and Instruments, and participation in CTR-USA, Inc. contractors meeting, NCI Smoke Inhalation Bioassay workshop and Tobacco Chemists Research Conference.

5. Miscellaneous (5,000). To include in-house analytical services, technical information services to prepare materials for publication and presentation, computing services to assist data management.

6. Unusual Costs (13,000).
   (a) Pressure transducer - for continuous measurement of puff volumes and dome pressure on SEM II (1,000).
   (b) Non-dispersive Infra-red Analyzer - for Carbon Dioxide Analysis on SEM animal containment unit (5,000).
   (c) Rapid response-time strip chart recorder - for use with item (a) and (b) (1,500).
   (d) Harvard Infusion - Pump - for sampling SEM and Walton exposure systems (1,500).
   (e) Sulfur and Nitrogen Specific Gas Chromatographic Detectors - for analysis of hydrogen cyanide and hydrogen sulfide (4,000)

Cost Summary

	Dollars
1. Salary and Overhead	$229,000
2. Materials and Supplies	16,000
3. Construction and Maintenance	20,000
4. Travel	7,000
5. Miscellaneous	5,000
6. Unusual Costs	13,000
	$290,000