ORIGINAL

PROTOCOL 1601.030

MBA Study Number: I-7009.030 Proposed Initiation Date: 11/2/84

Proposed Completion Date: 1/15/88

VALIDATION OF THE AMESA SMOKE GENERATION AND EXPOSURE SYSTEM

1.0 PURPOSE

The purpose of this study is:

a) to train personnel in the proper operation and maintenance of the machine;
b) to establish and calibrate the normal operating characteristics of the equipment; and
c) the characterization of tobacco smoke products of a reference cigarette.

2.0 SPONSOR

2.1 Name: Lorillard, Inc.

2.2 Address: Research Center
420 English Street
Greensboro, N.C. 27420

2.3 Authorized Representative: Thomas A. Vollmuth, Ph.D.
J. Daniel Heck, Ph.D.

3.0 TESTING FACILITY

3.1 Name: Inhalation Toxicology Section;
Microbiological Associates Inc.

3.2 Address: 5221 River Road
Bethesda, Maryland 20816

3.3 Study Director: Raymond M. David, Ph.D., D.A.B.T.

4.0 TEST CIGARETTE

4.1 Name: Reference III (Control)

4.2 Lot Number: 1493-86

88984366

MICROBIOLOGICAL ASSOCIATES INC.

4.3 MBA Test Article Number: T07009

4.4 Storage Conditions: Cigarettes will be stored at room temperature in their original cartons until use. Prior to use, all cigarettes will be conditioned for at least five days at 71±2°F and 60±10% humidity.

5.0 TEST SCHEDULE

5.1 Date Protocol Issued: September 9, 1987

5.2 Proposed Initiation Date: November 2, 1987

5.3 Proposed Completion Date: January 15, 1987

6.0 EXPERIMENTAL DESIGN

Personnel will be properly trained in the use of the machine by a representative of AMESA. Following this training, a period of experimentation will begin in which various parameters will be changed in order to get a "feeling" for the proper operation of the machine. Appropriate flow meters, etc., will be calibrated. Reference cigarettes will be loaded onto the machine and various nominal concentrations generated. During exposures, samples of the aerosol will be taken for determination of particle size distribution, total particulate matter (wet) and dry particulate matter corrected for nicotine and water. Carbon monoxide will also be measured. No animals will be used under this protocol.

6.1 Concentrations: A variety of nominal concentrations (0.5, 1.0, 3.0, 6.0 and 10.0%) will be attempted. These concentrations will be based on a two second puff and a 35 ml puff volume. Concentrations will be varied by adjusting dilution air.

6.2 Smoke Exposure Regimen: All exposures will be dynamic with flow rates adjusted to the manufacturers suggestions, but may be altered at the discretion of the study director or sponsor.

6.3 Analyses and Measurements:

6.3.1 Gravimetric samples: Gravimetric concentrations of total particulate matter will be determined using glass fiber (Cambridge) filters. Weights will be corrected for nicotine and water content using a method specified by the sponsor. Samples for determination of concentrations will be withdrawn from the breathing zone of the animals. At least three measurements will be taken per nominal concentration at each tier.

6.3.2 Particle Size Determination: Cascade impactors will be used to separate and/or quantitate various categories of particle sizes from the exposure module of animals. At least three samples per tier will be taken and analyzed per exposure concentration.

6.3.3 Carbon monoxide determinations: Filtered samples of the gas phase will be taken and analyzed for carbon monoxide levels using a carbon monoxide gas analyzer. At least three measurements will be taken per exposure concentration per tier.

7.0 METHODS

7.1 Total Particulate Matter: Gravimetric determinations will be made by pre-weighing a 45 mm diameter glass-fiber filter (Cambridge filter, Cambridge Filter Corp., Syracuse, NY), placing the filter in a filter holder, and connecting the filter to the sampling port of the exposure module. Air samples will be drawn through the filter using a vacuum supply regulated by a flow meter. Flow rates are normally set at 0.5 L/min and a sampling time of 4-8 min is normally used. The concentration of aerosol present in the chamber was calculated by the difference in weight of the filter divided by the volume of air sampled. Weights will be corrected for nicotine and water content by a method to be supplied by the Sponsor.

7.2 Particle Size Determination: Particle size determinations will made using a Mercer 7 stage cascade impactor with cutoff stages of 4.5, 3.0, 2.1, 1.5, 1.0, 0.7 and 0.3 micron particles with a final filter after the last stage. Preweighed glass coverslips will be placed onto each stage and the impactor assembled and attached to the exposure module. Air samples will be drawn through the impactor using a vacuum supply. The flow rate is normally 1 L/min and is controlled by a critical orifice. The difference in weight of each stage and the cumulative percentage of the total weight of each stage is calculated. The Mass Median Diameter is determined by the stage that contained the median cumulative weight based on a linear representation of the data. The net weight gain of each stage and the total weight collected will also be calculated.

7.3 Carbon Monoxide Determination: The carbon monoxide (CO) levels in the gas phase of the smoke aerosol will be determined at appropriate intervals using a carbon monoxide analyzer (supplier to be determined). Carbon monoxide levels will be measured at least twice for each smoke concentration.

8.0 EVALUATION OF TEST RESULTS

The following will be presented in the final report.

8.1 Draft SOP's for the "start-up" and operation of the smoking equipment will be written.

8.2 Values for "dry" particulate matter vs. the percentage of smoke will be presented. Variations in values based on the tier position will be noted.

8.3 Particle size distribution vs. the percentage of smoke will be presented. Mass Median Diameter for each concentration will be calculated. Variations based on the tier position will be noted.

8.4 Levels of carbon monoxide and nicotine in the smoke will be presented for each concentration tested.

8.5 All methods used in the conduct of this study will be described.

9.0 RECORDS AND SAMPLE ARCHIVES

9.1 Records: Upon completion of the final report, copies of the raw data will be sent to the Sponsor and the originals will be retained by MBA for a reasonable period of time. The Sponsor will be notified of any disposition of data.

9.2 Cigarettes: Cigarettes not utilized will be stored in a locked cabinet at room temperature. The Sponsor will be consulted regarding final disposal.

10.0 STANDARD OPERATING PROCEDURES AND PROTOCOL ALTERATION

All procedures not specified in this protocol will be performed in accordance with the Microbiological Associates Inc. Standard Operating Procedures Manual and in compliance with the EPA Good Laboratory Practice Standards.

11.0 SIGNATURES

Sponsor Approval — Date 11-19-87

Study Director — Date 12/16/87