

THE FILE

1

AD _____

INHALATION TOXICOLOGY OF RED AND VIOLET DYE MIXTURES

PHASE I: ENGINEERING REPORT

Prepared by:
David W. Davies

Mark A. Higuchi
NSI-ES Technology Services Corporation
Research Triangle Park, NC 27709

JUNE 1989

Principal Investigator: Daniel L. Costa, Sc.D.

Supported by:

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701

Army Project Order 87PP7808

Pulmonary Toxicology Branch
Environmental Toxicology Division
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Project Officer: Mr. James C. Eaton, PE
Health Effects Research Division
U.S. ARMY MEDICAL BIOENGINEERING RESEARCH AND DEVELOPMENT LABORATORY
Fort Detrick, Frederick, MD 21701

Approved for public release;
distribution unlimited

The findings of this report are not to be construed as
an official Department of the Army position unless so
designated by other authorized documents.

AD-A211 666

DTIC
ELECTE
AUG 11 1989
S D CS D

AD _____

INHALATION TOXICOLOGY OF RED AND VIOLET DYE MIXTURES

PHASE I: ENGINEERING REPORT

Prepared by:
David W. Davies

Mark A. Higuchi
NSI-ES Technology Services Corporation
Research Triangle Park, NC 27709

JUNE 1989

Principal Investigator: Daniel L. Costa, Sc.D.

Supported by:

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701

Army Project Order 87PP7808

Pulmonary Toxicology Branch
Environmental Toxicology Division
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Project Officer: Mr. James C. Eaton, PE
Health Effects Research Division
U.S. ARMY MEDICAL BIOENGINEERING RESEARCH AND DEVELOPMENT LABORATORY
Fort Detrick, Frederick, MD 21701

Approved for public release;
distribution unlimited

The findings of this report are not to be construed as
an official Department of the Army position unless so
designated by other authorized documents.

Accession For	
NTIS CRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and/or Special
A-1	



FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

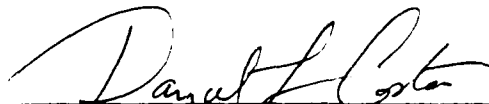
_____ Where copyrighted material is quoted, permission has been obtained to use such material.

_____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

☒ Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

☒ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

_____ For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

 6/24/89

Daniel L. Costa, Sc.D. Date
Principal Investigator

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.		
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE					
4. PERFORMING ORGANIZATION REPORT NUMBER(S)			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION ITD, HERL U.S. Environmental Protection Agency		6b. OFFICE SYMBOL (if applicable)	7a. NAME OF MONITORING ORGANIZATION U.S. Army Biomedical Research and Development Laboratory		
6c. ADDRESS (City, State, and ZIP Code) Research Triangle Park, NC 27711			7b. ADDRESS (City, State, and ZIP Code) ATTN: SGRD-UBG-M Fort Detrick, Frederick, MD 21701-5010		
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research and Development Command		8b. OFFICE SYMBOL (if applicable) SGRD-ACQ	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Project Order No. 87PP7808		
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5012			10. SOURCE OF FUNDING NUMBERS		
			PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.
			WORK UNIT ACCESSION NO.		
11. TITLE (Include Security Classification) Inhalation Toxicology of Red and Violet Dye Mixtures Phase I: Engineering Report					
12. PERSONAL AUTHOR(S) David W. Davies & Mark A. Higuchi					
13a. TYPE OF REPORT Phase Report		13b. TIME COVERED FROM 3/15/87 TO 11/1/88		14. DATE OF REPORT (Year, Month, Day) 1989, June	
15. PAGE COUNT					
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	RA3, Animal toxicology exposure facility, aerosol, inhalation, aerosol generation, anthraquinone dye mixtures, containment facility.		
19. ABSTRACT (Continue on reverse if necessary and identify by block number)					
An inhalation exposure facility was developed at the U.S. Environmental Protection Agency, Research Triangle Park, NC, to conduct inhalation exposures of rodents to dye mixtures used by the U.S. Army in the manufacture of smoke munitions. The exposures were to evaluate the health effects of inhaled dye mixtures on rodents and simulate industrial-type exposures in load, assembly, and pack plants. Workers at the plants can accidentally come in contact with the dye, by dermal and/or pulmonary exposures. (Cont'd)					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian			22b. TELEPHONE (Include Area Code) 301-663-7325		22c. OFFICE SYMBOL SGRD-RM1-S

The exposure facility is designed to keep the dye from spreading into surrounding areas and to isolate this study from other toxicological studies being performed in the same research facility. The facility consists of an exposure laboratory and a foyer. The foyer includes a small room used to conduct analytical work, a shower, and clothes-change facility. Ingress and egress for the exposure laboratory are through a small vestibule in the foyer. Personnel entering the exposure laboratory wear shoe covers, Tyvek® suit, gloves, head cover and particle filter mask.

Exposures are conducted in four Hazelton 2000 stainless steel, whole-body, live-in, exposure chambers. Three of the chambers, each with independent concentration control, are used for dye exposures. The fourth chamber is used as a filtered air control. Concentrations of dye mixtures have been generated from 140 to 800 mg/m³ with mass median aerodynamic diameters of approximately 2.75 micrometers with a geometrical standard deviation (σ_g) of 2.25. Preliminary chamber characterization test atmospheres were generated for 6 hours per day, which is the maximum length of an exposure proposed for the toxicological study.

EXECUTIVE SUMMARY

The U.S. Army and the U.S. Environmental Protection Agency (EPA) entered into an interagency agreement to study the health effects of inhalation exposures of dye mixtures on laboratory rodents. The dye mixtures are utilized by the U.S. Army in the manufacture of colored smoke munitions (M18 grenades). The U.S. Army Biomedical Research and Development Laboratory provided funding to the Pulmonary Toxicology Branch of the Environmental Toxicology Division of the Health Effects Research Laboratory of EPA at the Research Triangle Park, NC, to conduct toxicological evaluations of laboratory rodents exposed to the dye mixtures by inhalation and dermal contact. This engineering report describes the facility developed to conduct inhalation exposures of laboratory rodents to the dye mixtures.

Each of the two dye mixtures to be studied is a binary formulation of two dyes. Formulation and mixing of the dyes was performed by the U.S. Army Chemical Research Development and Engineering Center, Munitions Directorate, Production Division, Aberdeen Proving Ground, MD. The red grenade mixture was formulated by combining the anthraquinone dye Disperse Red 11 (DR11) and the azo dye Solvent Red 1 (SR1); the violet grenade mixture was formulated from DR11 and Disperse Blue 3 (DB3), another anthraquinone dye. For field use, additional components are incorporated into the mixture to enhance the burning properties. During actual grenade loading, assembly, and packing processes, plant personnel may be incidentally exposed, by inhalation or dermal contact, to the dye mixtures. Toxicological studies with laboratory rodents are designed to simulate both types of exposures. Although dermal studies will be

conducted, this report describes only the facilities used for inhalation exposures.

The exposure laboratory was developed as a containment facility in an effort to restrict the contamination of adjacent exposure and research facilities with the dye powders. The exposure facility is designed with permanent access through an entry foyer. A sophisticated ventilation system is designed to contain the dye powder within the confines of the exposure laboratory. Animals will be housed in one of four live-in inhalation chambers for the duration of their exposure regimen. Simultaneous dye exposures will be conducted in three chambers, each having a different concentration. The fourth chamber will serve as the filtered air control.

The U.S. Army funded a study at the Inhalation Toxicology Research Institute (ITRI), Lovelace Biomedical and Environmental Research Institute in Albuquerque, NM, to develop a method for generating respirable size particles of two dye mixtures, a yellow dye and a yellow/green dye.¹ ITRI selected a jet mill style generator after testing several different units. Aerosol atmospheres ranging from approximately 20 to 250 mg/m³ were generated at ITRI. Mass median aerodynamic diameters (MMAD) from 3.0 to 5.4 μ m with a geometric standard deviation (σ_g) of approximately 2.0 were obtained. Results from this study were used to select an aerosol generator for the EPA's tests.

After preliminary consideration of alternative generation systems, it was decided that a jet mill (Jet-O Mizer, Model 0101, Fluid Energy Processing and Equipment Company, Hatfield, PA) provided the most practical means of generating adequately dispersed concentrations of the dye aerosols. The jet

mill is an opposing air-jet grinder/generator which, as a result of the high air velocities necessary for proper operation, produces considerable noise across the frequency spectrum. Exposure chamber systems were evaluated for sound level under a variety of conditions and it was determined that appropriate noise abatement for the safety of operating personnel as well as for study animals was necessary. Sound dampening structural improvements in the generator enclosure and interruptions in the jet mill to chamber connecting conduits produced significant reductions in full spectrum noise. This is particularly important for those frequencies that produce high rodent stress levels.

An evaluation of chamber aerosol homogeneity was conducted to determine the uniformity and reproducibility of the concentration and particle size of dye aerosol throughout the breathing zone of the test animals. Results of the distribution test are discussed in this report.

Three dyes, DR11, SR1, and DB3, were chemically analyzed for purity. The bulk red and violet dye mixtures were analyzed for composition. Results of these analyses have been submitted to the U.S. Army in quarterly reports.² Future chemical analyses will be reported in this manner. They will also be summarized in the reports on the results of the inhalation exposures.

The animal inhalation exposure facility, developed under the interagency agreement, is designed to permit a complete regimen of inhalation tests. A study protocol developed for these tests proposed 6-hour acute, and 28- and 90-day subchronic exposures. Both subchronic exposures will be for 6

hours/day x 5 days/week. Aerosol concentrations for the 28- and 90-day exposures would be determined after reviewing the acute testing results. Data generated during the studies will be used to evaluate the health effects by inhalation of the dye mixtures in laboratory rodents.

ACKNOWLEDGMENT

Support services for this study were provided by NSI-ES Technology Services Corporation under EPA Contract Number 68-02-4450. This work was conducted in response to Technical Directives 4.1.2 (Engineering), 4.1.3 (Chemistry), and 4.3.2 (Animal Care).

Major contributions were provided by James Andrews and John Bobrowski of EPA, Leon C. Walsh III, Bob Jones, John McKee and Michael Hiteshew of NSI-ES Technology Services Corporation.

TABLE OF CONTENTS

	<u>Page</u>
FOREWORD	
EXECUTIVE SUMMARY	1
ACKNOWLEDGMENT	5
LIST OF FIGURES	7
LIST OF TABLES	8
 INTRODUCTION	 9
 FACILITY DESCRIPTION	 12
Isolation Facility	12
Laboratory Environmental Systems	14
Laboratory Control	14
Exposure Chamber	18
Compressed Air System	22
Vacuum System	22
Chamber Exhaust Filtration System	26
Disposal	28
Waste Water Treatment System	29
Collection	29
Treatment	29
Disposal	31
 EXPOSURE FACILITY OPERATIONS	 32
Dye Generation System	32
Aerosol Monitoring	38
 RESULTS AND DISCUSSION	 41
Isolation Room	41
Aerosol Concentration and Particle Size	41
Aerosol Chamber Distribution	44
Air and Water Filtration	45
Facility Utilization	46
 LITERATURE CITED	 47
 DOCUMENT DISTRIBUTION LIST	 48
 APPENDIX A: CHEMICAL STRUCTURES OF DYES	 49
 APPENDIX B: STANDARD OPERATING PROCEDURES	 50
 APPENDIX C: PROTOTYPE CHAMBER DISTRIBUTION WITH RED DYE MIXTURE	 110
 APPENDIX D: NOISE ANALYSIS	 116
 APPENDIX E: FILTER DATA SHEET	 125

LIST OF FIGURES

	<u>Page</u>
1. Exposure laboratory and foyer floor plan	13
2. Air systems	15
3. Laboratory HVAC schematic	16
4. Humidity sampling system	19
5. Chamber dilution air supply system	21
6. Compressed air system	23
7. Vacuum system	25
8. Chamber and hood exhaust system	27
9. Waste water treatment schematic	30
10. Exposure system - front view	33
11. Exposure system - side view	34
12. Aerosol generation system	36
13. Generator impactor	37
14. Aerosol sampling system	40
15. Mass median aerodynamic diameter for red grenade mixture	43
B-1. Bacterial analysis forms	97
B-2. Animal identification card	107
C-1. Sample probe locations for chamber distribution test	111
C-2. Distribution sampling system.	113
D-1. Baseline jet mill noise test	119
D-2. Jet Mill noise test	120
D-3. Baseline noise test	122
D-4. Baseline noise with modifications	124

LIST OF TABLES

	<u>Page</u>
B-1. Aerosol monitoring record	73
B-2. Particle size distribution form	77
C-1. Distribution Data	114
C-2. Summary statistics	114
E-1. Filter data table	125

INTRODUCTION

The U.S. Army manufactures smoke grenades which are filled with mixtures of particulate organic dyes and a number of combusting or oxidative elements which yield a profuse colored smoke upon ignition. These smoke grenades are used for signaling and marking purposes by the U.S. Army. The dye is purchased from commercial suppliers, formulated into combustible mixtures and placed within grenades in load, assembly, and pack (LAP) plants. The nature of these dye compounds makes full containment difficult during grenade manufacture and hence incidental worker exposure is difficult to avoid. Contact may be via inhalation or dermal exposure. To assess the potential health effects of inhalation or dermal exposures, the U.S. Army has funded an Interagency Agreement (#RW21932489-01-0) with the Toxicology Branch, Inhalation Toxicology Division of the Health Effects Research Laboratory, U.S. Environmental Protection Agency (EPA), Research Triangle Park, NC. A small animal inhalation exposure laboratory has been constructed to conduct whole body exposures of rodents to binary mixtures of the dyes for periods of up to 90 days.

Each of the two dye mixtures which will be studied contains two of three dyes with minor amounts of associated impurities. The red dye is a formulation of Disperse Red 11 (DR11) and C.I. Solvent Red 1 (SR1) (Lot CRDEC 88F24-01); and the violet dye mixture is a formulation of DR11 and Disperse Blue 3 (DB3) (Lot CRDEC 88F27-01). The DR11 is 1,4-diamino-2-methoxyanthraquinone (Appendix A). SR1 is 1-[(2-methoxyphenyl)azo]-2-naphthalenol (Appendix A). DB3 is 1-[(2-hydroxyethyl)amino]-4-(methylamino)-anthraquinone (Appendix A). Other nomenclature is also used for the dyes; Solvent Violet 26

for DR11, Sudan R or Sudan Red G for SR1 and C.I. Disperse Blue 41 for DB3. In the M18 grenade, additional combustion and oxidative constituents are added but these ingredients are not included in the dye mixtures to be used for animal testing.

An isolation suite consisting of an exposure laboratory, an entrance foyer, and an analytical laboratory, was installed within the Air Toxics Exposure Facility of the Environmental Research Center, Research Triangle Park, NC, for the conduct of animal inhalation exposures to dye compounds. The exposure laboratory was designed to contain the dye powder. Facilities and procedures were developed to minimize spreading of the dye powder, both in bulk and aerosolized forms during operation and maintenance of the exposure system. The exposure laboratory was constructed to house four whole-body, live-in exposure chambers. The generation system simultaneously provides three of the chambers with three different aerosol concentrations. The fourth chamber serves as a filtered air control. The aerosol generation system is controlled manually. One senior operator and an animal care/technical assistant conduct all exposures. Standard operating procedures for operating the exposure laboratory are described in Appendix B. The exposure laboratory is designed to provide animal exposures for 6 hours/day for regimes of 1 day, 4 weeks, and 3 months. Animals will live in the exposure chambers for the duration of each study and will only be removed from the laboratory when each exposure protocol is completed. Shoebox-type rat cages with filter tops will be used to transport the test animals to the appropriate researcher's laboratory for toxicological evaluation. A separate toxicology report will be issued to the U.S. Army at the completion of the project.

Chemical analysis procedures have been developed for the assessment of purity, composition, and quantification of constituents of the bulk dye mixtures and filter samples collected from the exposure chambers during aerosol generation. These methods and results have been presented to the U.S. Army in EPA quarterly reports.² Chemistry data are not included in this engineering report.

This is the final report describing the completion of Phase 1 of the project: Development of an inhalation exposure facility to conduct exposures of rodents to dye mixture aerosols.

FACILITY DESCRIPTION

ISOLATION FACILITY

The dye isolation exposure laboratory was developed by renovating existing space and installing a prefabricated isolation room within an existing high-bay area. The dye laboratory is located in room J216 of the EPA's national air pollution research facility, the Environmental Research Center (ERC). The ERC is located in the Research Triangle Park, North Carolina.

The exposure facility, constructed to conduct the dye exposures, is shown in Figure 1. The exposure laboratory is 392 ft². Entrance to the laboratory is through a 135 ft² foyer complex, consisting of a clothes-change/shower-out room, short hallway airlock, and a small analytical laboratory. Egress from the exposure laboratory is through the same rooms in the reverse direction. Personnel leaving the laboratory are required to shower to remove personal dye accumulations and to minimize risk of contamination of surrounding areas. Outer garments are removed at egress and placed in receptacles for incineration at a later time.

The foyer was constructed by renovating existing space using standard construction techniques and materials. The exposure laboratory was constructed by installing a pre-engineered modular room or Unilab (HEMCO Corporation, Independence MO) in an existing high-bay. The walls and ceiling of the Unilab were fabricated of 3 in. thick urethane foam faced with enameled

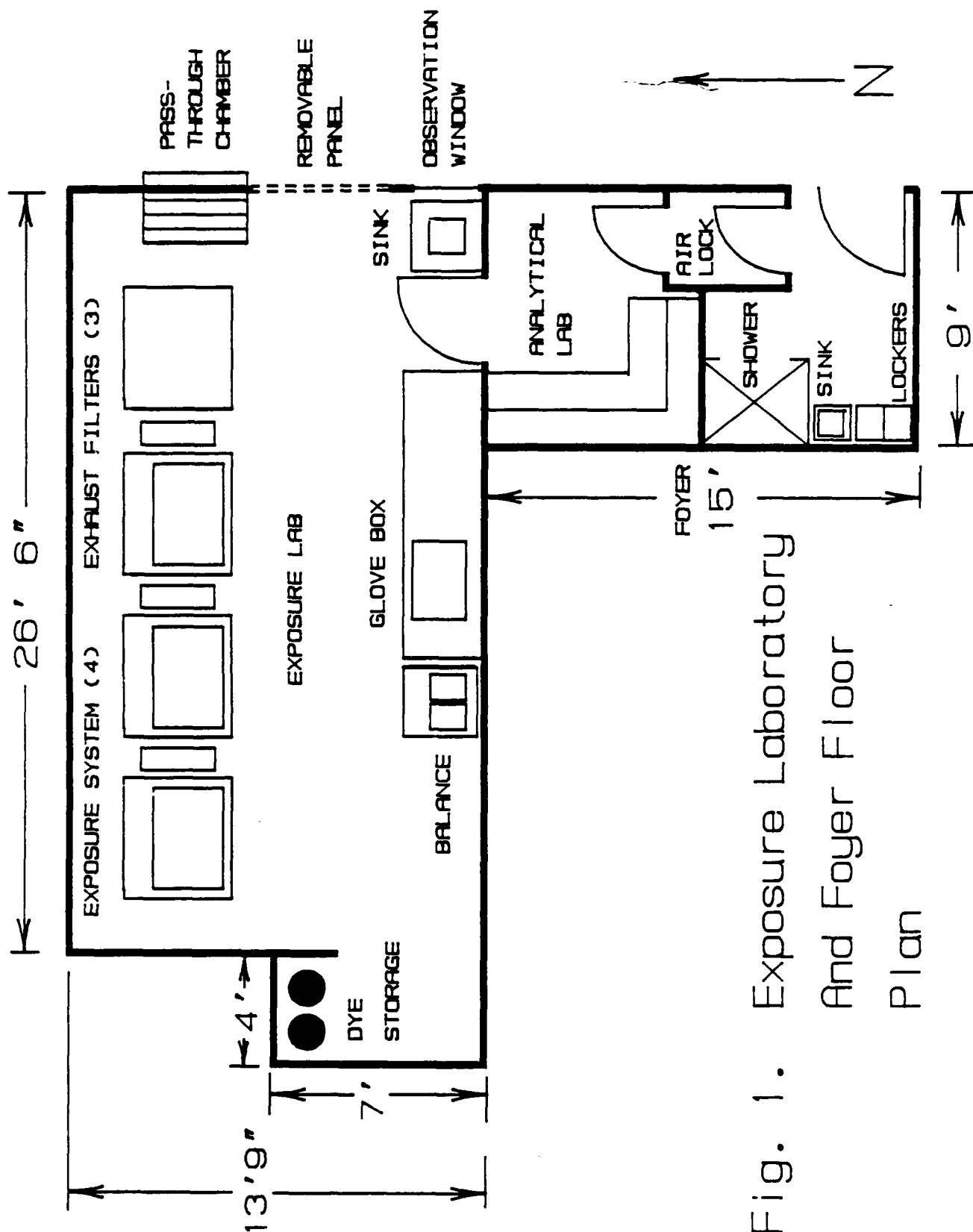


Fig. 1. Exposure Laboratory
And Foyer Floor
Plan

steel. The ceiling is approximately 12 ft high. A storage and handling alcove for the barrels of bulk dye is located in the southwest corner of the exposure laboratory.

To transfer large equipment and chambers into and out of the exposure laboratory, a removable panel was installed in the east wall. While the exposure laboratory is in operation, the removable panel is secured and sealed. Animals will be transferred into and out of the exposure laboratory by way of a pass-through, also located in the east wall.

LABORATORY ENVIRONMENTAL SYSTEMS

A dedicated containment laboratory was constructed to provide a safe, secure working environment for conducting the animal inhalation exposures to dye aerosol. This laboratory contains and is serviced by several major mechanical systems illustrated in Figure 2. In this section, two main environmental control systems are described; one for the laboratory and a second that provides the chamber dilution air.

Laboratory Control

Laboratory heating, ventilation, air conditioning (HVAC), and filtration, shown in Figure 3, are provided by a 7.5 ton split system air conditioner (Model BTA090C300MB, TRANE Co., Fort Smith, Az. 72903) with electric heat and duct mounted electric steam boiler (Model X-15B, Autoflo, Detroit, Mi. 48239).

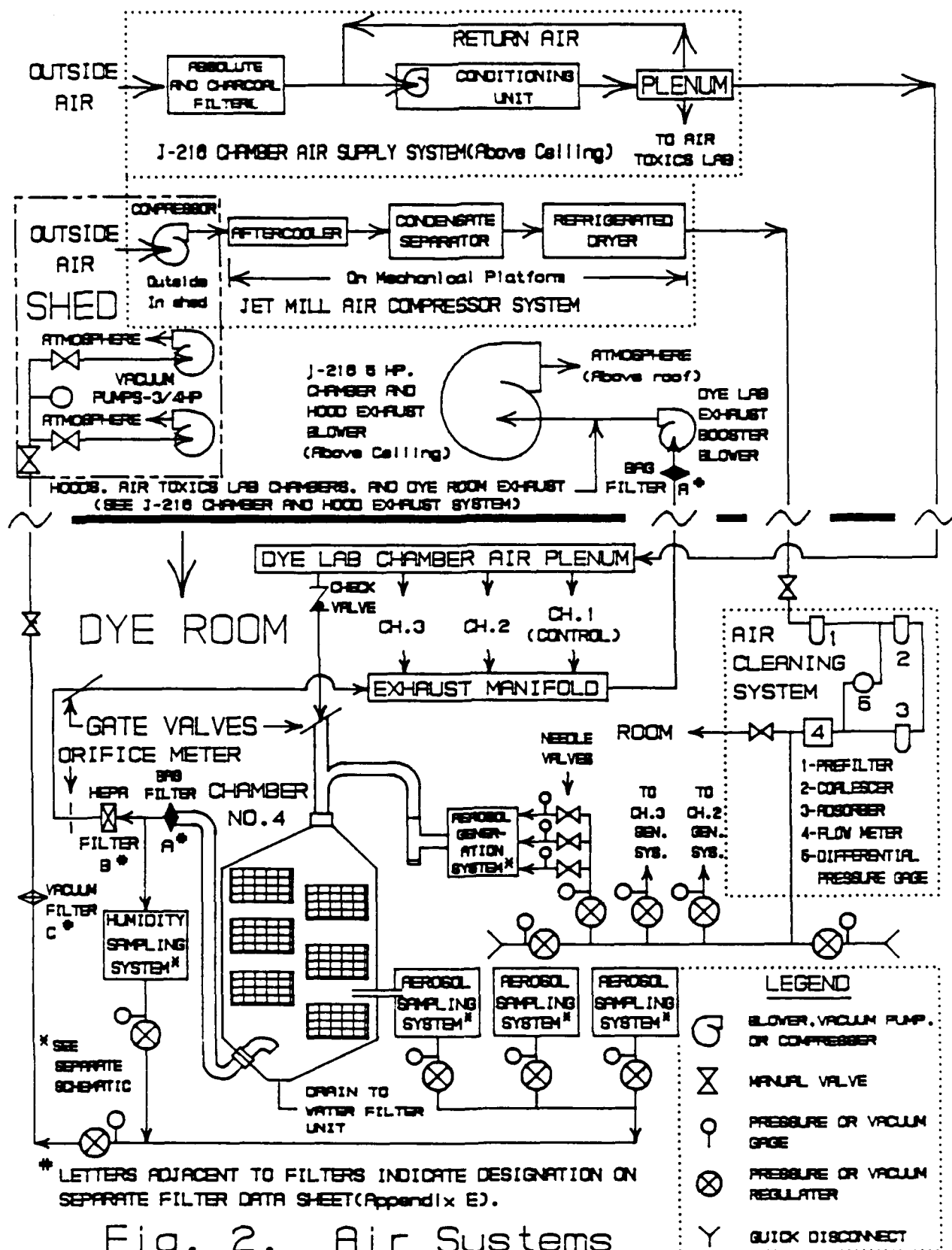
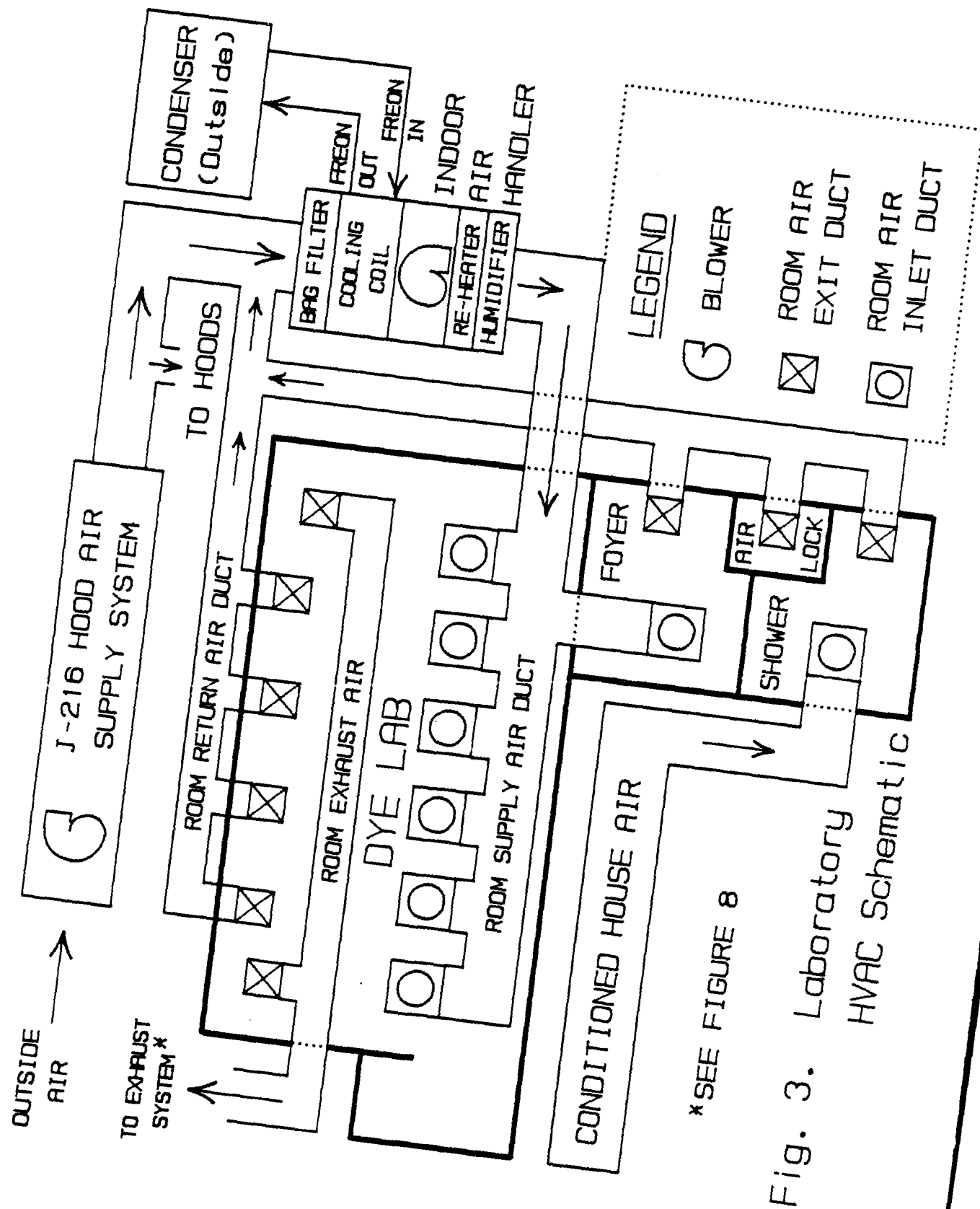


Fig. 2. Air Systems



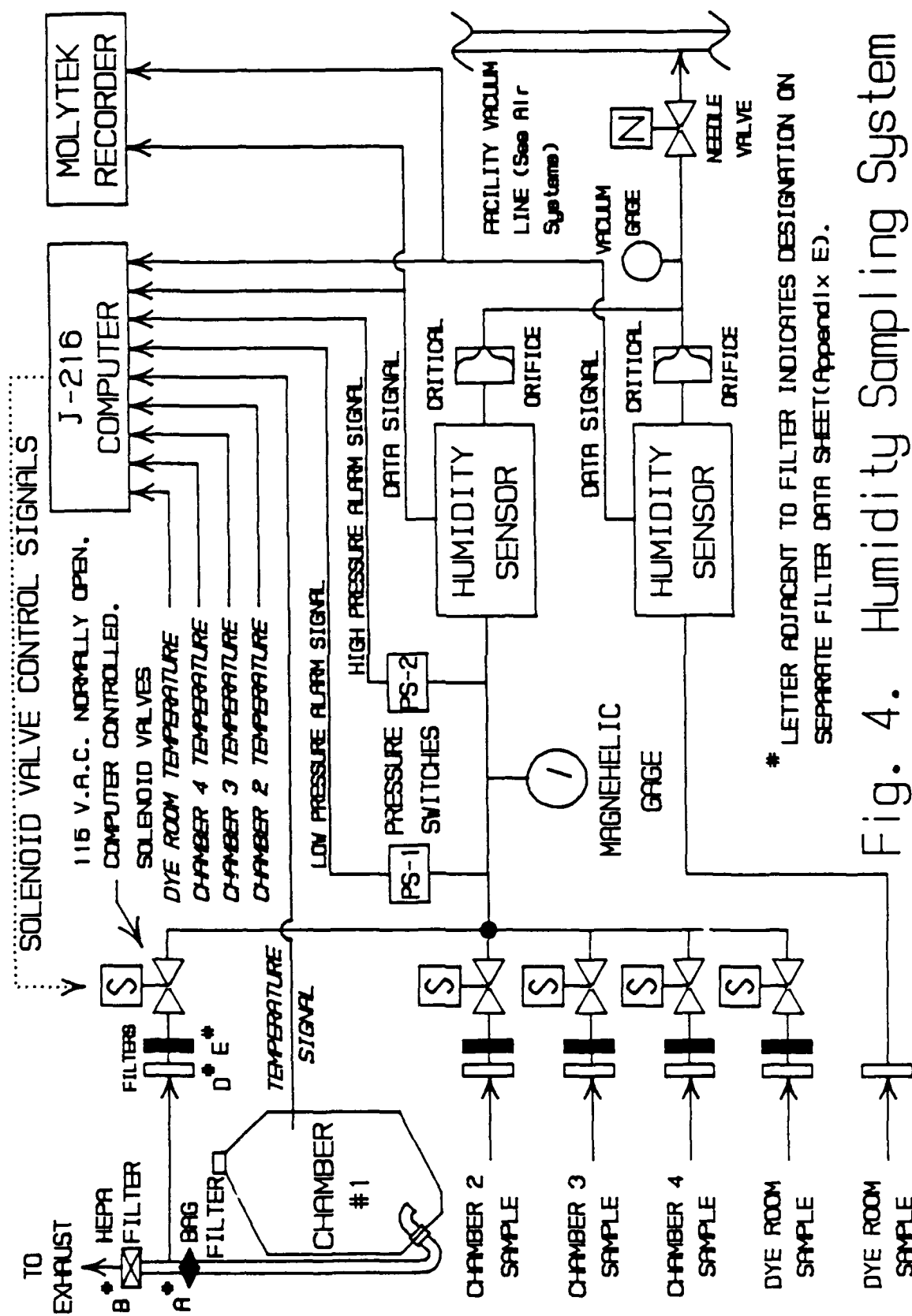
This system recirculates 3000 cubic feet per minute (cfm) filtered (Model 9T24-21 bag filter, Filtration Technology Inc., Greensboro, N.C. 27419) air through the dye laboratory and foyer area. Approximately 600 cfm outside air is continuously drawn into the return air duct upstream of the bag filter housing. The excess system air (600 cfm) is exhausted through 2 vents located in the ceiling and at opposite ends of the laboratory providing 10 air changes per hour in the laboratory. Supply air diffusers and return air grilles are positioned to direct a continuous flow of conditioned air around the chambers. This air also helps regulate the temperature inside the exposure chambers. Volumetric control dampers, installed in all ducts, assure control of the flow of conditioned air throughout the laboratory. A wall mounted thermostat and humidistat are located to provide quick response to changes in the room conditions. These electric controls are wired in parallel with a process control computer (Model ADAC System 1200, ADAC Corp., Woburn, Ma. 01801) located in an adjoining laboratory.

Electronic temperature sensors are installed within each chamber and near the laboratory thermostat. Signals from the temperature sensors are directed to a strip chart recorder/data logger located in the foyer and to the process control computer located in an adjacent laboratory. Two chilled mirror-type, dewpoint hygrometers (Model 880, EG&G International, Inc., Waltham, MA) continuously monitor the exposure chambers and laboratory dewpoint levels. One hygrometer monitors the exposure laboratory only and the other, through a computer controlled, multiplexed valving panel, monitors the four chambers and the exposure laboratory. Relative humidities in the laboratory and exposure chambers are calculated from measured dewpoint and dry bulb temperatures. A schematic of the relative humidity monitoring/control system is depicted in

Figure 4. This monitoring is controlled by the process control computer. Samples are drawn through each sample line, sequentially, at 5 minute intervals. Signals from the two dewpoint hygrometers are sent to the datalogger and the process control computer. Three modes of operation are possible with the control setup: (1) automatic control of the temperature and relative humidity in the laboratory, using the control computer to regulate the mechanical systems; (2) thermostatic control of the room temperature only; and (3) continuous operation of the cooling coil for dehumidification, with mechanical control of temperature and relative humidity. Precise control of the exposure laboratory temperature is critical to the maintenance of environmental conditions in the animal chambers.

Exposure Chamber

The exposure laboratory contains four stainless steel/glass exposure chambers (Model 2000, Hazleton Systems, Aberdeen, MD) assembled to allow simultaneous aerosol exposures in three chambers while the fourth chamber is used as a filtered air control. The 2 m³ chambers are fitted with automatic watering systems, removable feed troughs and excreta catch pans. Each chamber is designed with three tiers of two cage modules with catch pans under each tier. Each cage module contains sixteen individual cages sized for extended housing of rats. This caging configuration can house up to thirty two animals per shelf. The design of these exposure chambers makes use of the excreta pans to aid in distribution of the test aerosol.^{3,4} Since the experimental protocol for the planned dye studies calls for a maximum of thirty two animals



* LETTER ADJACENT TO FILTER INDICATES DESIGNATION ON SEPARATE FILTER DATA SHEET(Appendix E).

Fig. 4. Humidity Sampling System

for any one exposure, only the middle tier of cages will be used. Excreta pans will be inserted above and below the cages to assure chamber performance. (See distribution tests results in Appendix C).

Air is supplied to the exposure chambers from two sources. Approximately 300 liters per minute (lpm) of filtered, dried air is supplied to the jet mill. Air from the jet mill particle generator is mixed with filtered, pre-conditioned dilution air to provide a continuous 500 lpm flow through the chamber. Breathing quality air is supplied to the jet mill aerosol generator from a remote air compressor. Dilution air is supplied by a conditioned air loop which serves two laboratories simultaneously. This system is shown in figure 5. Outside air is drawn in through a throttling valve, a high efficiency particulate air (HEPA) filter and a charcoal filter and mixed with plenum return air. This air is then conditioned to the desired temperature and relative humidity. Conditioned air is circulated in an insulated plenum maintained at a positive pressure of approximately 0.50 in. H₂O gauge. Dilution air is supplied to the chambers from this plenum. Volume control valves and check valves allow control of the dilution air supplied to each chamber.

The combination of conditioned dilution air and controlled laboratory air temperature maintains stable and precise conditions in the exposure chambers. Redundancy in the environmental control systems allow for continued operation of the laboratory should one component fail during subchronic testing.

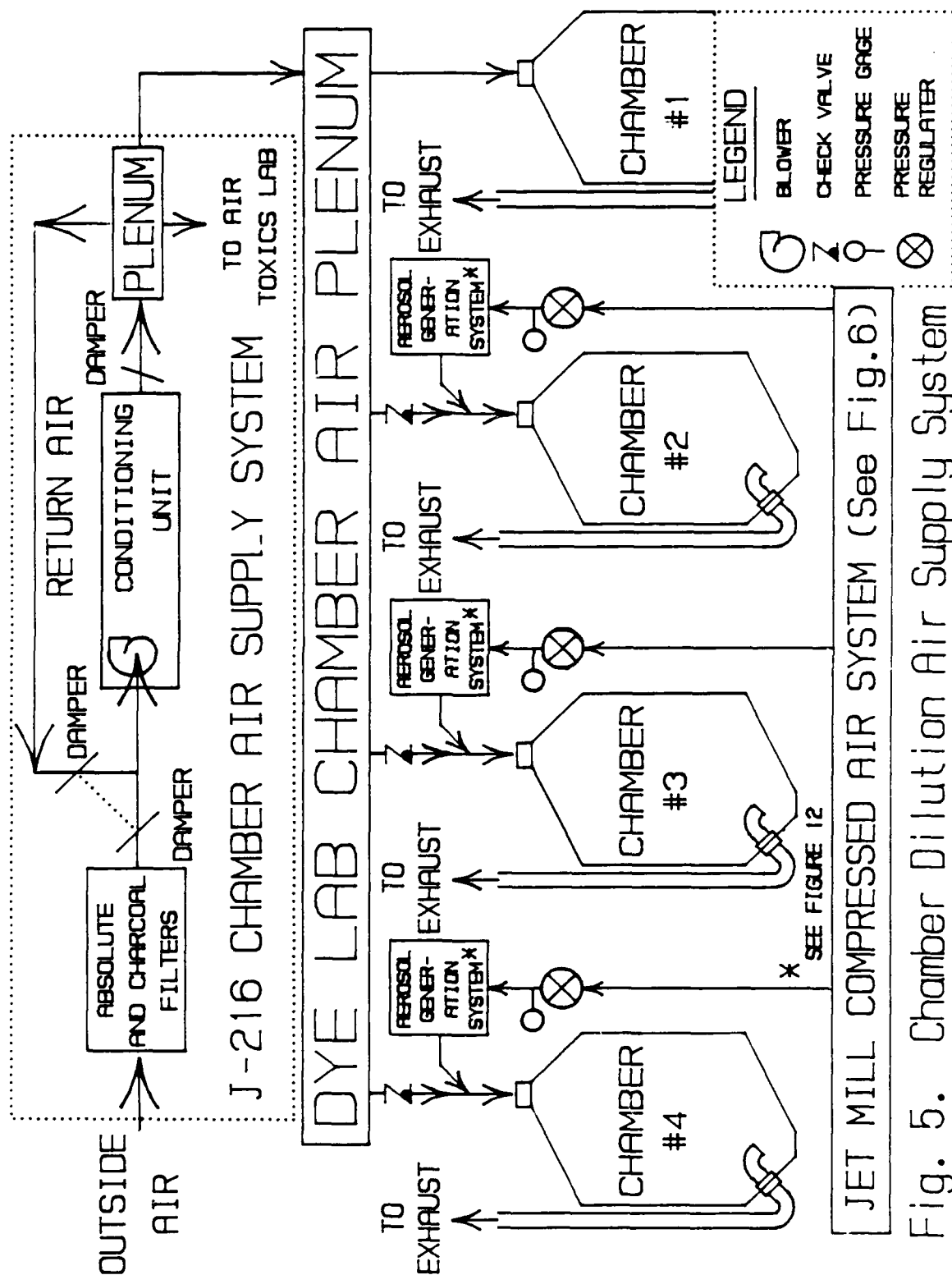


Fig. 5. Chamber Dilution Air Supply System

Compressed Air System

Compressed air is utilized during dye exposures by the jet mill aerosol generators. Inside the generator, high pressure air is delivered at high velocity causing the dye particles to impact on themselves creating the grinding action. Milling of the particles is controlled by the pressure of the air supplied to the jet mill. Normal operating range for each of the generators is approximately 10 cfm of air at 40-90 psig. Since existing laboratory systems did not have this capacity, a system, shown in Figure 6, was installed to provide 60 standard cubic feet per minute of filtered, dry compressed air at 90-100 psig.

The compressed air system consists of a 15 hp screw-type compressor, with aftercooler, condensate separator, and a refrigerated dryer (Model TA-015T, Joy Manufacturing, PA). Additional filters (Appendix E) installed downstream of the compressor remove entrained oil and provide breathing quality air to operate the generators.

Vacuum System

Vacuum is required in the exposure laboratory to collect samples for determination of aerosol concentration, aerosol particle size, and relative humidity in the exposure chambers and in the isolation laboratory. A dedicated vacuum system was installed to meet the needs of the laboratory.

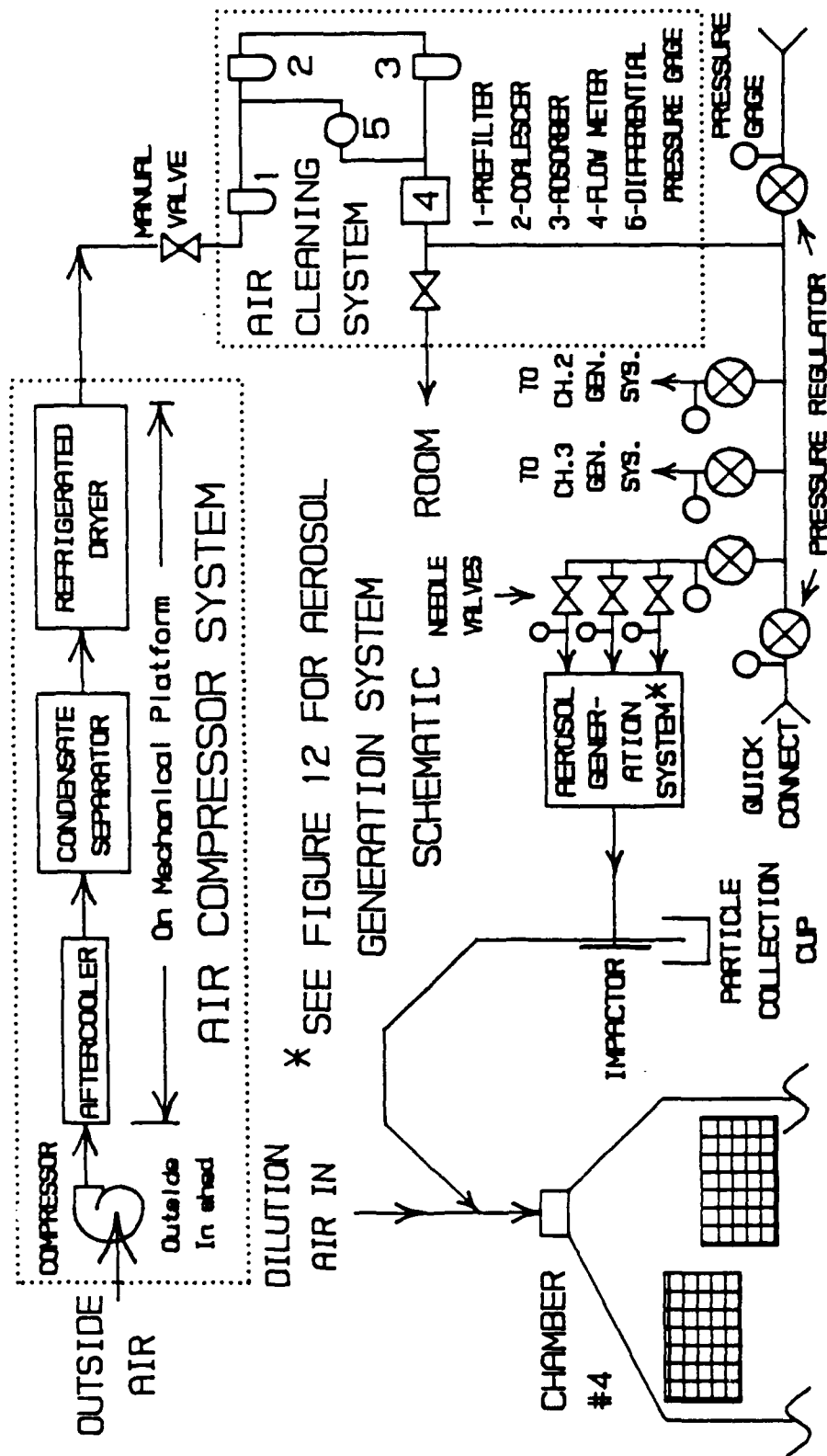


Fig. 6. Compressed Air System

The system, shown in Figure 7, and included along with other facility air systems illustrated in Figures 2 and 3, consists of two 0.75 hp vacuum pumps (Model 5VSF-10-M508X, Gast Manufacturing Corporation, Benton Harbor, MI) and associated valves, filters, regulators and gauges.

Both vacuum pumps are located outside the ERC building in a shed which also houses the air compressor for the dye facility. The system's capacity is sufficient to maintain a vacuum of greater than 26 in. mercury with the three aerosol sampling systems and the relative humidity system operating, or more than 20 in. of Hg. with a cascade impactor operating at 1 cfm.

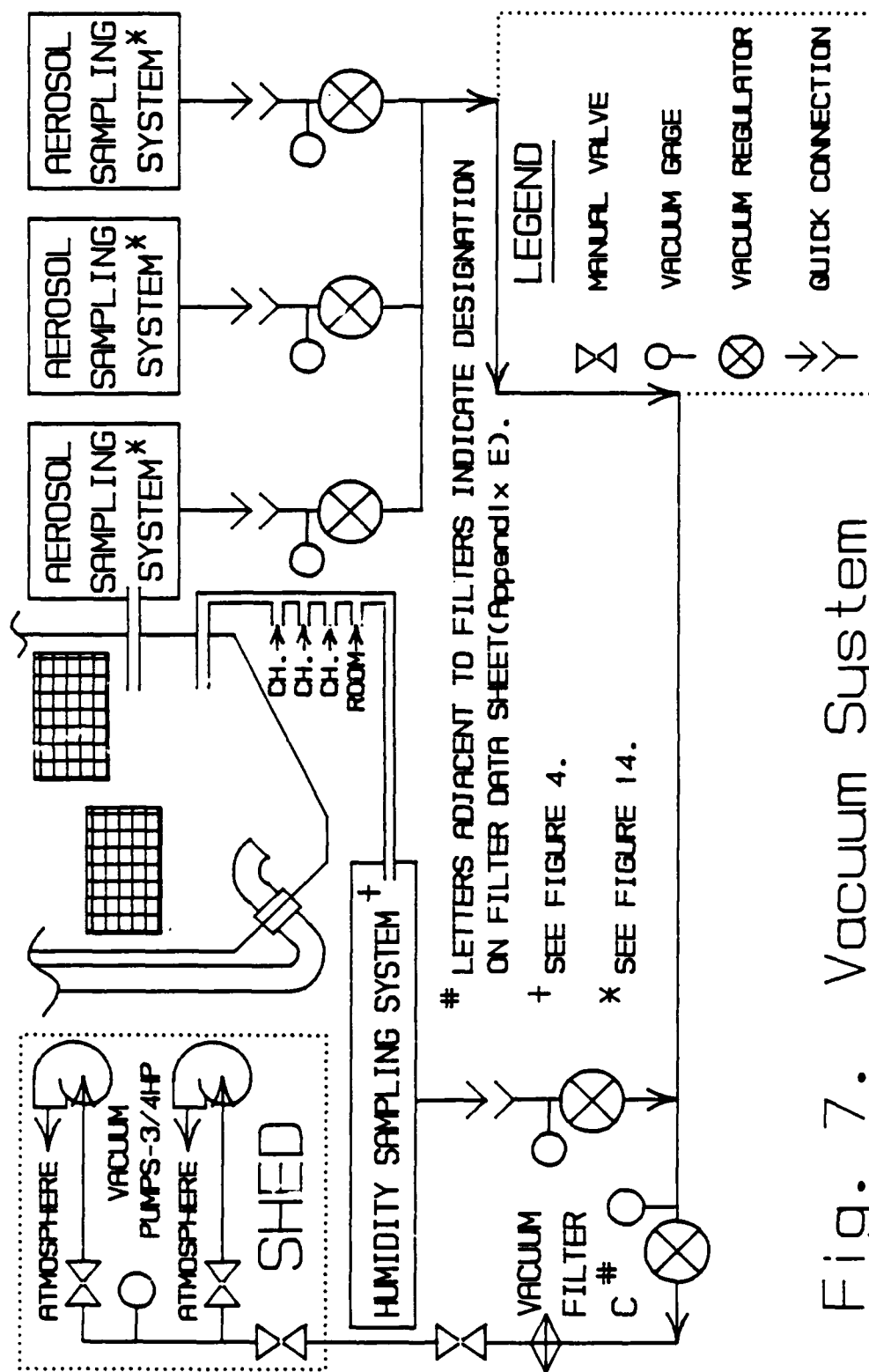


Fig. 7. Vacuum System

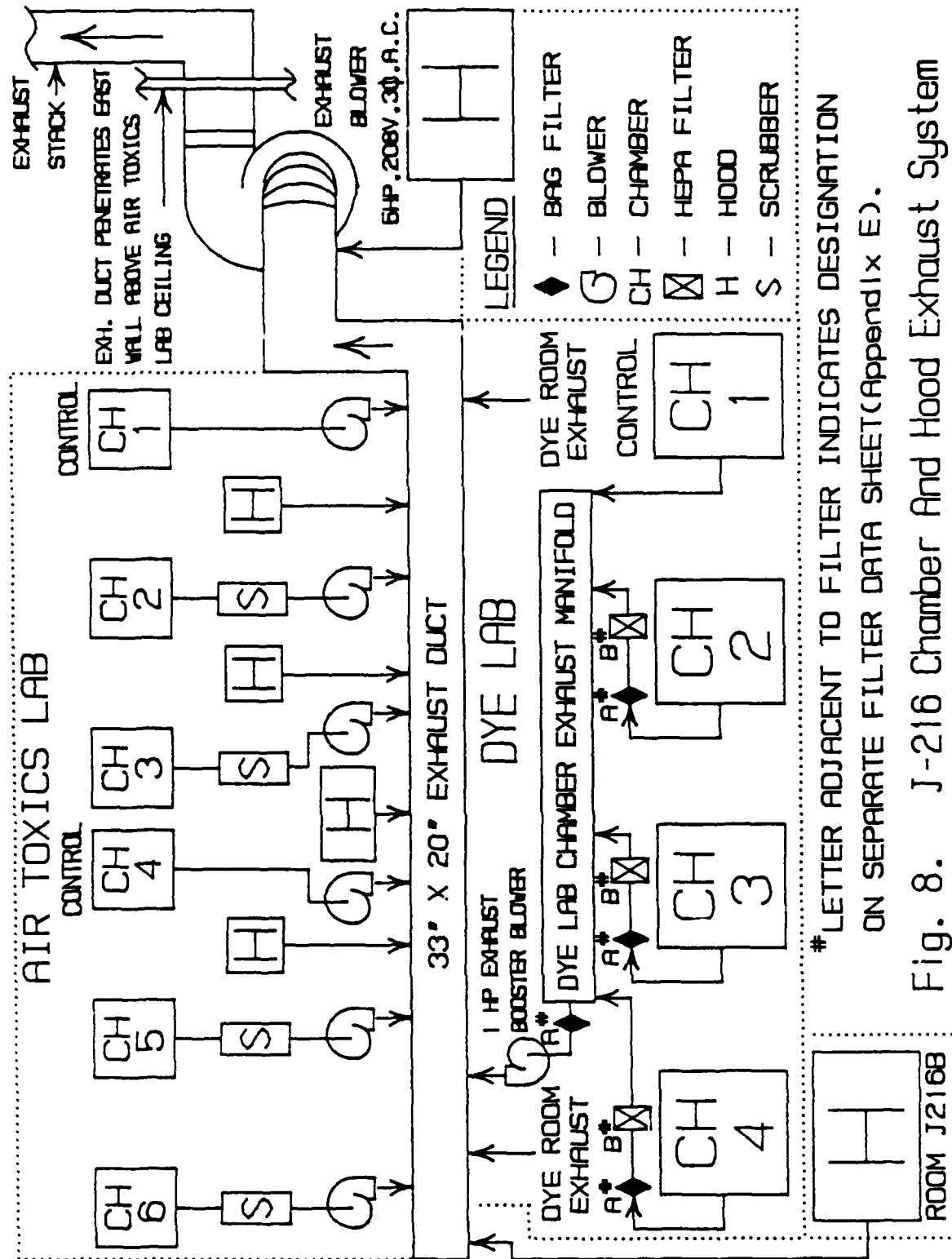
CHAMBER EXHAUST FILTRATION SYSTEM

A large volume of dye particles is contained in the exposure chamber effluent. These residual dye particles are removed prior to chamber exhaust air entering the laboratory exhaust system. A chamber exhaust system, Figure 8, was designed with the safety of laboratory personnel and minimal contamination of existing laboratory equipment as primary requirements.

The chamber exhaust system uses a roughing filter (bag type, model 9T24-21, Filtration Technology Inc., Greensboro, N.C.) followed by a HEPA filter (Appendix E) for removal of dye particles from the exhaust air stream. Air from all of the exposure chambers is funneled into a common exhaust manifold. A second roughing filter is located in the duct connecting the exhaust manifold with the laboratory exhaust system.

Roughing filter housings were constructed of transparent plexiglass and fitted with a removable top for bag filter installation and removal. The plexiglass allows visual inspection of the bag for leaks or tears. The roughing filter, rated to remove up to 95% of 1 micron particles (Appendix E) filters the majority of the dye particles.

HEPA filters are self-contained and are mounted in-line, downstream of the bag filters in the exhaust duct of each chamber. The HEPA filters, rated at 99.97% removal of 0.3 micron particles (Table E), remove any remaining dye particles.



* LETTER ADJACENT TO FILTER INDICATES DESIGNATION ON SEPARATE FILTER DATA SHEET (Appendix E).

Fig. 8. J-216 Chamber And Hood Exhaust System

Disposal

Once the bag filters are loaded with dye particles, indicated by the pressure drop across the filters reaching -0.5 in. H₂O gauge, the filters are removed from the plexiglass housing. The bag filter is double bagged into plastic liners and boxed for off-site incineration.

Due to the high efficiency of the bag filters, the HEPA filter is expected to last for the duration of the exposures. If the HEPA filters need replacement, they will be handled in the same manner as the bag filters.

WASTE WATER TREATMENT SYSTEM

Collection

Contaminated water generated in the dye laboratory by chamber sanitation and sonication of the powder feeder parts is collected in 18 gallon holding tanks (four) recessed in the floor of the laboratory. The dye water is then transferred to 15 gallon drums by a pump mounted in each holding tank. The outside of each drum is decontaminated and the drums are transferred to another laboratory for treatment by a waste water treatment unit (Model 1M3, Heavy Metal Removal Process Electrochemical Unit, ANDCO Environmental Processes, Inc., Amherst, NY). The waste water treatment system is illustrated in Figure 9. This unit is located in room Q-218 of the EPA, ERC.

Treatment

Dye contaminated water is processed by the "Andco Color Removal Process". This is a patent pending electrochemical process that is designed to reduce the levels of dye particles in waste water from the fabric industry. A custom built unit was adapted for cleanup of waste water generated during operation and maintenance of the dye exposure facility.

Operationally, ferrous ions are added to process water inside the cell (ANDCO) by passing the wastewater stream between cold-rolled steel plates, which function as the "electrodes" in the electrochemical process. A DC

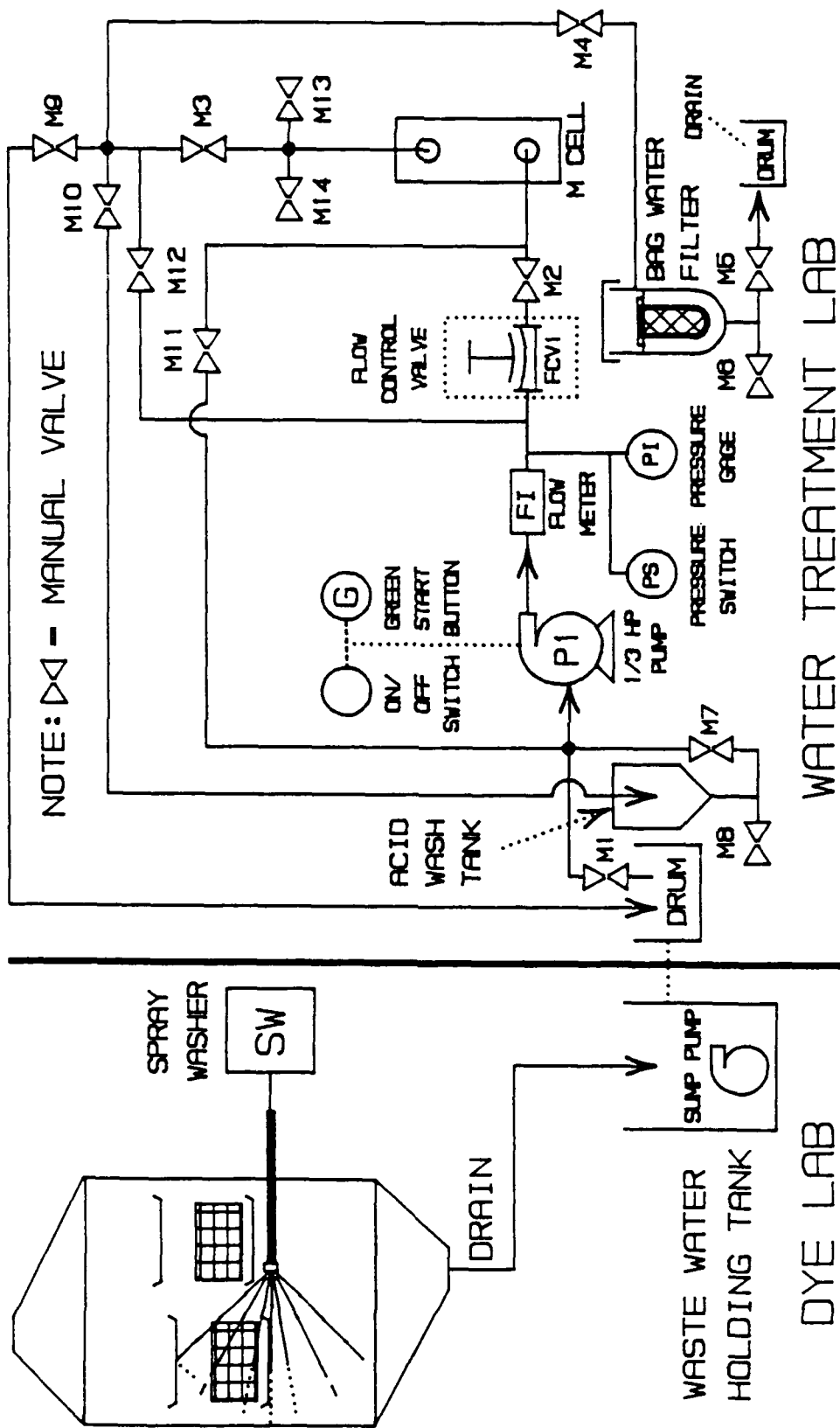


Fig. 9. Waste Water Treatment Schematic

current is applied across the electrodes, causing a small amount of water to break down into hydrogen gas and hydroxyl ions with the simultaneous cogeneration of ferrous ions. The stream exiting the cell is essentially a water solution containing iron hydroxide. The iron hydroxide, with adsorbed dye molecules attached, is removed from the water by a bag filter. This process is repeated until the resulting stream has a non-detectible quantity of dye. The detectible quantity of dye in water is >2 ppm as determined by visible light spectroscopy.

Disposal

Treated water is disposed of directly into the sewer. The bag filter containing the iron hydroxide and dye is removed, bagged and boxed for offsite incineration.

EXPOSURE FACILITY OPERATIONS

DYE GENERATION SYSTEM

The dye generation system includes a jet mill grinder/generator, dry powder material feeder with hopper (AccuRate, Model 106, AccuRate, Inc.), single stage impactor, sound dampening conduit, stainless steel tubing (2.5 in. and 3 in.), solenoid, timer and generation system containment box. There are two complete generation systems in operation: one for the highest concentration chamber and one for the two lower concentration chambers. The generator orientation is depicted in Figures 10 and 11.

Dye material (either red or violet grenade mixture) is delivered to the jet mill from the bulk powder feeder. The quantity of dye delivered by the powder feeder is controlled by increasing or decreasing the helix revolution rate. This delivery rate is not uniform for the dye mixtures; therefore a modified push/pull solenoid, activated by a timer, strikes the nozzle containing the helix at regular time intervals. This action prevents the dye from packing between the nozzle and helix and provides more consistent dye delivery to the jet mill.

The powder feeder delivers the dye powder through a venturi to the jet mill grinding chamber, where two high speed air jets grind the bulk powder material into finer particles. Once the particles reach a certain size, determined by the jet mill's operating parameters, they exit the classifier outlet at a high velocity. The resulting particle size of the dye material is a

LEGEND

- 1) CHAMBER DILUTION AIR
- 2) GENERATOR SILENCER WITH RADIOACTIVE ELEMENT
- 3) DRY POWDER FEEDER
- 4) AEROSOL OBSERVATION WINDOW WITH RADIOACTIVE ELEMENT
- 5) JET MILL
- 6) AEROSOL INJECTION LINE
- 7) IMPACTOR
- 8) LARGE PARTICLE COLLECTION CUP
- 9) JET MILL AIR PRESSURE CONTROL VALVES AND GAUGES
- 10) DRY POWDER FEEDER CONTROLLER
- 11) H-2000 EXPOSURE CHAMBER
- 12) ANIMAL EXPOSURE CAGE
- 13) BAG FILTER AND HOUSING
- 14) EXHAUST LINE
- 15) GENERATOR CONTAINMENT

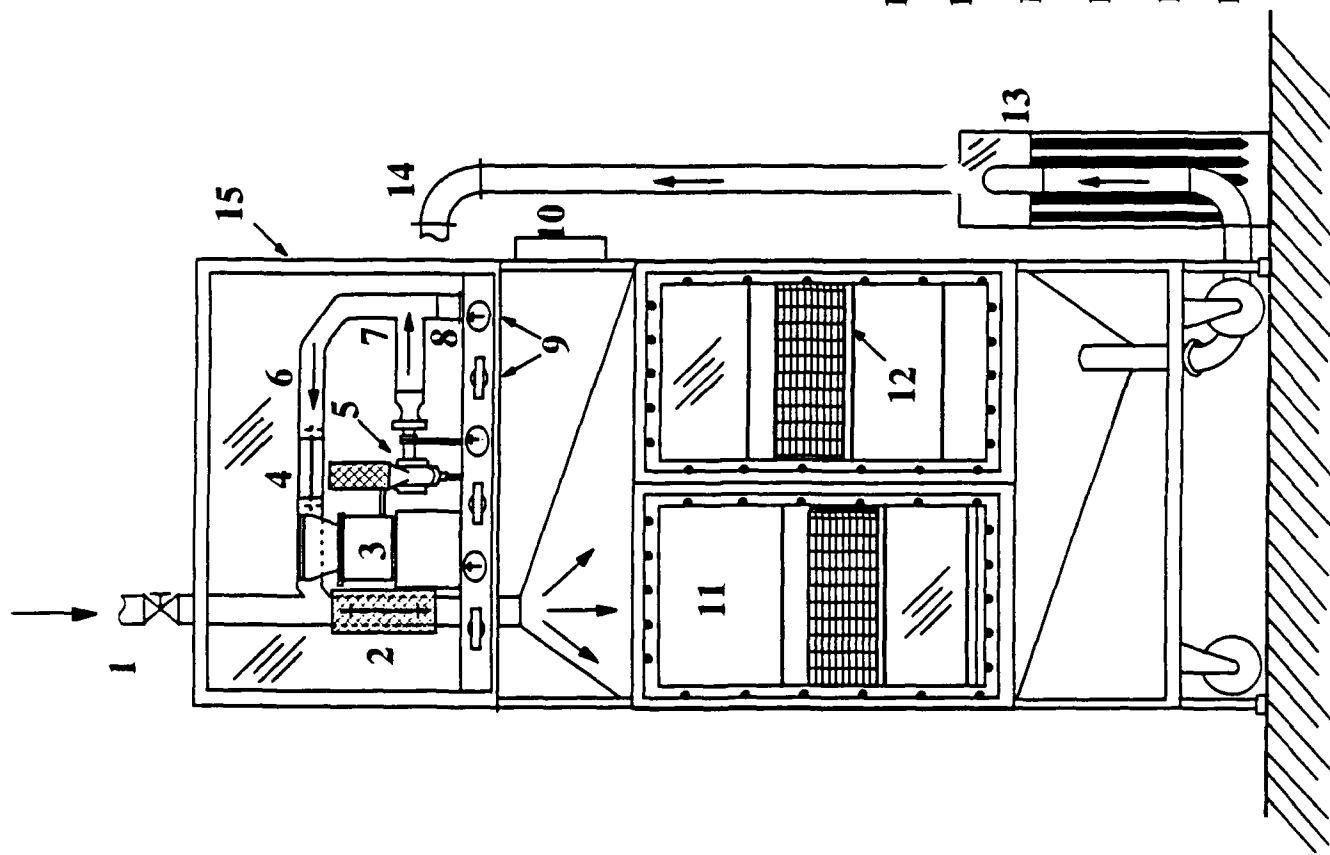
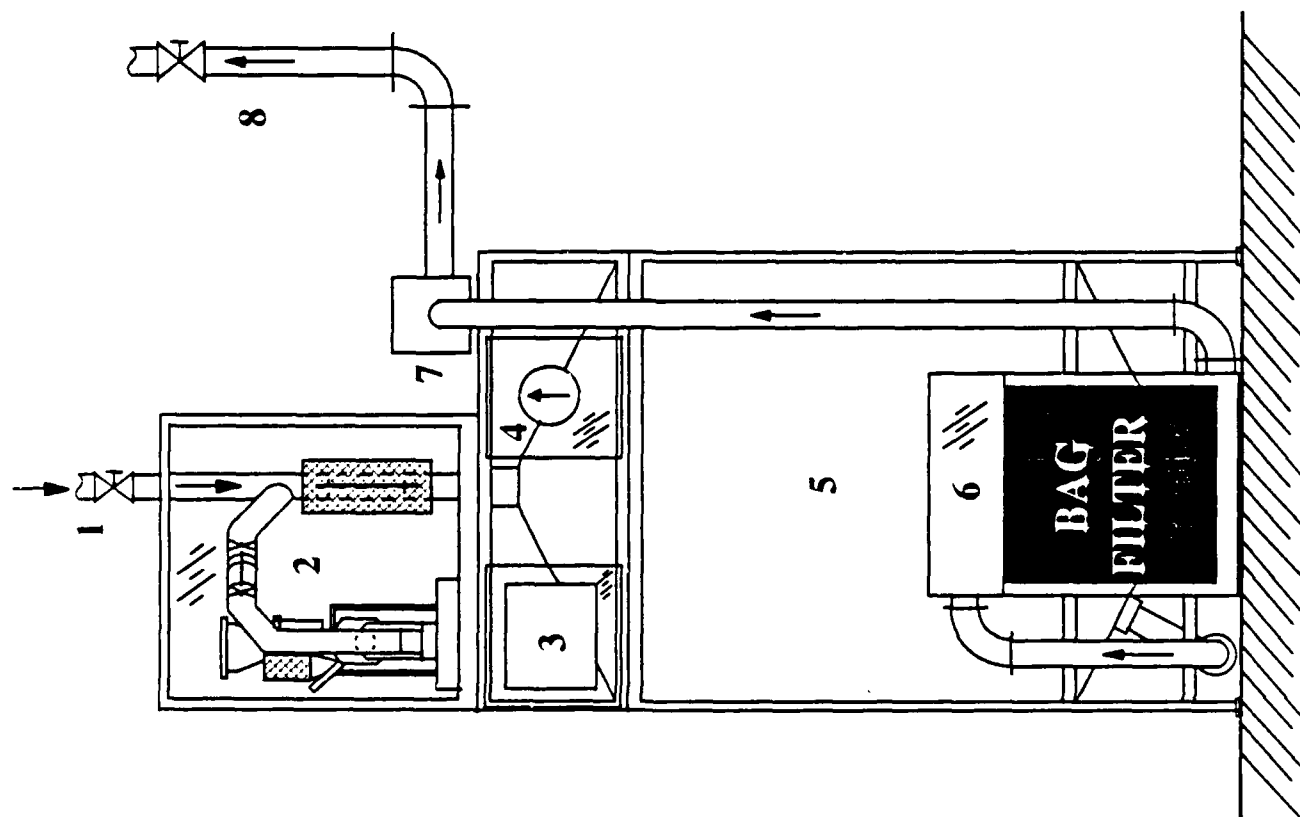


Figure 10. Exposure system - front view.



LEGEND

- 1) CHAMBER DILUTION AIR
- 2) AEROSOL GENERATOR
- 3) DRY POWDER FEEDER CONTROLLER
- 4) MAGNEHELIC VACUUM GAUGE
- 5) ANIMAL EXPOSURE CHAMBER
- 6) BAG FILTER AND HOUSING
- 7) HEPA FILTER
- 8) EXHAUST LINE

Figure 11. Exposure system - side view.

function of the amount of dye introduced into the jet mill and the operating pressure of the jet mill. The higher the pressure used to operate the jet mill, the larger the particle size generated. The generation system is illustrated in Figure 12.

The ground dye exits the jet mill and enters a single stage impactor, shown in Figure 13, where the particles are accelerated toward a hard impaction surface. Inertia carries the larger particles to the impaction surface, while the aerodynamically smaller particles remain entrained in the air stream entering the exposure chamber. The dye aerosol is carried through stainless steel tubing (2.5 in.) to the stainless steel chamber air supply tubing (3 in.). The air stream of dye aerosol is mixed with humidified dilution air prior to entering the exposure chamber. Before entering the chamber, aerosol passes through a tubular section of sound dampening foam (3 in. dia. by 1.5 in. thick by 1 ft. long). This foam reduces both high and low frequency noise created by the jet mill. (The experimental methods and results of the noise monitoring study are described in appendix D.) One each, 10 in., 10 mCi Kr85 radioactive source is mounted in the observation window (transparent conduit) and the foam sound-deadening conduit in the aerosol stream to eliminate electrostatic charge on the dye particles.

The jet mill aspirator cup, which pulls dye into the jet mill, is fitted with a similar section of sound insulating foam that reduces the amount of noise transmitted to the laboratory.

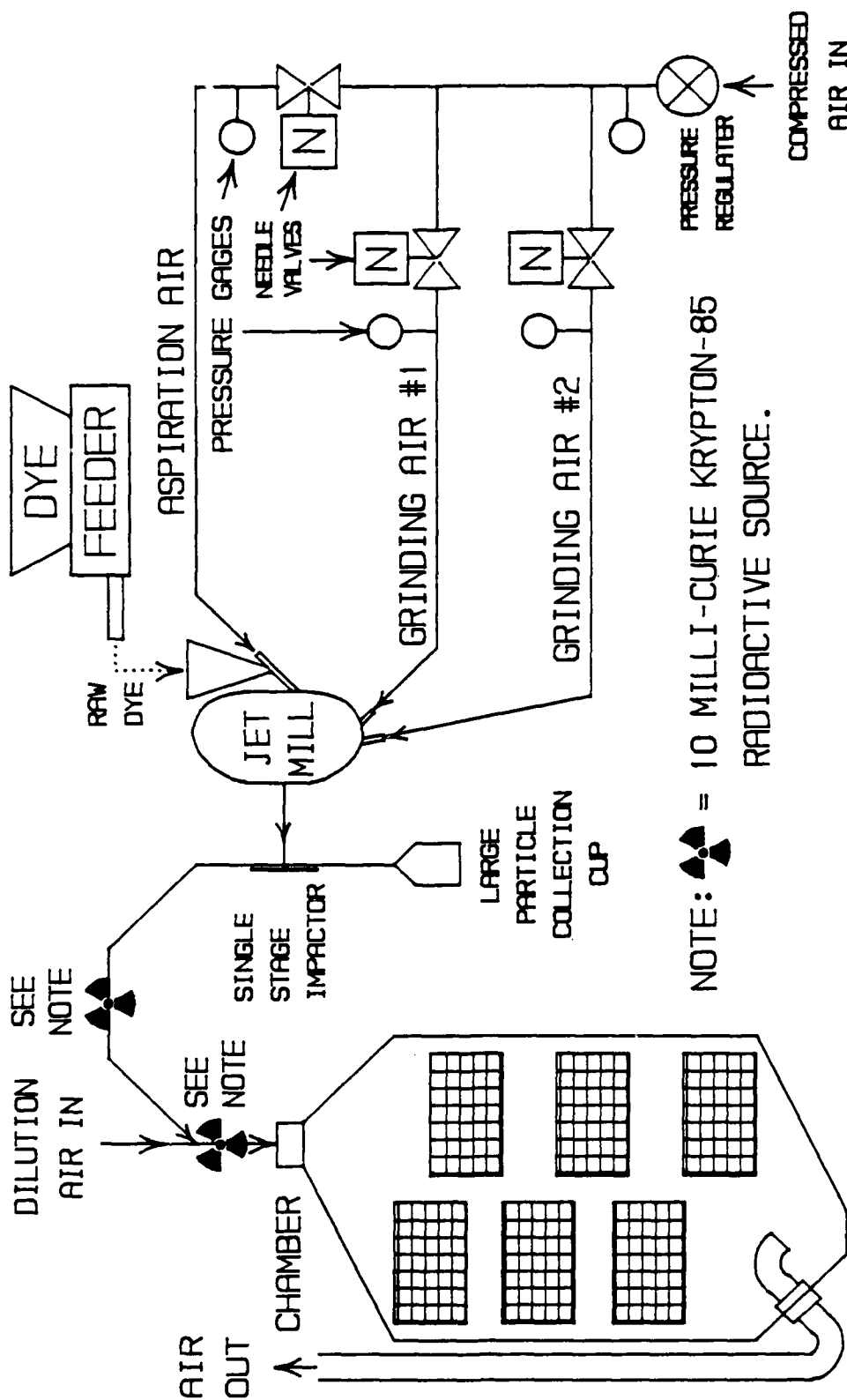


Fig. 12. Aerosol Generation System

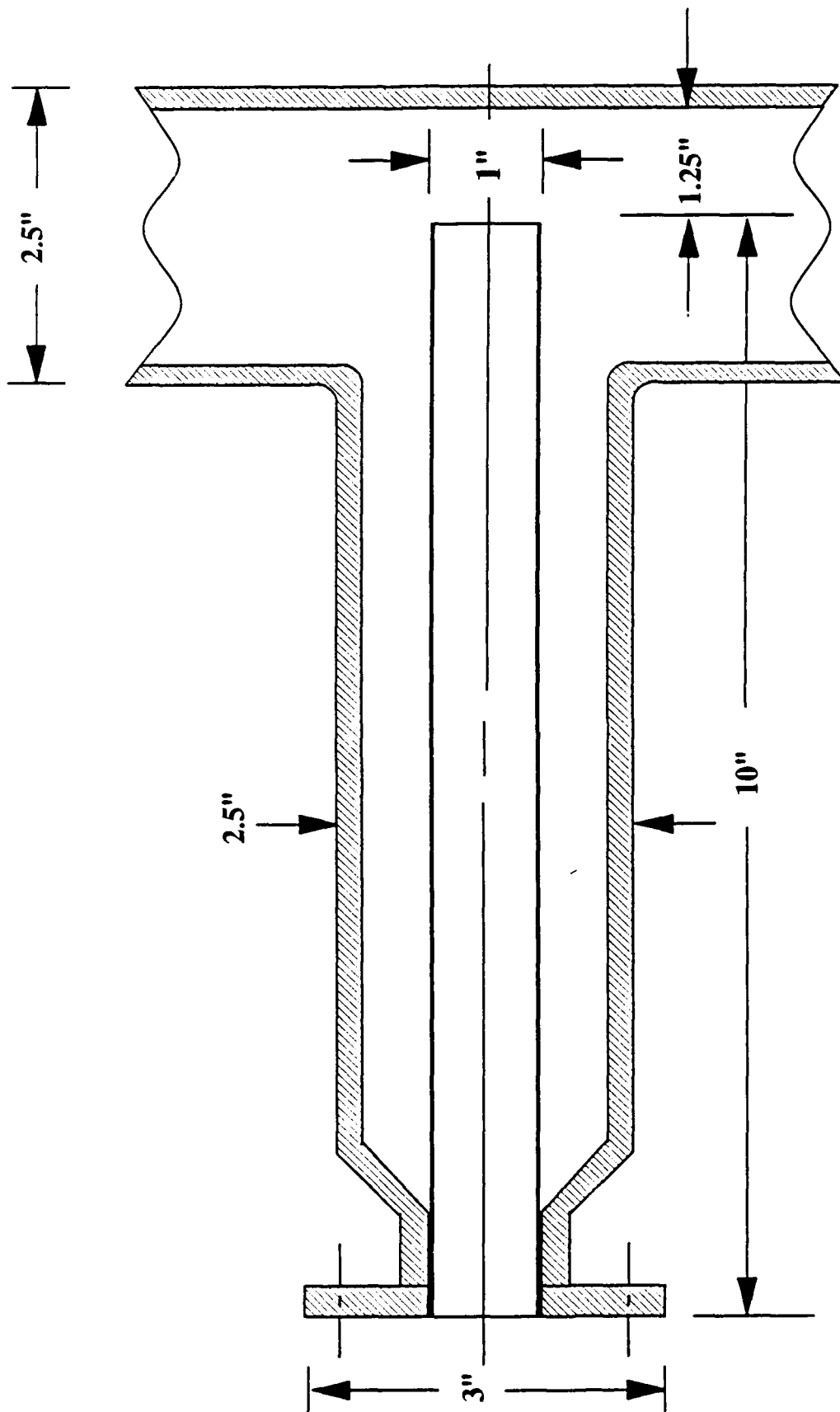


Figure 13. Generator impactor.

The entire generation system is enclosed in a Uvex® and steel reinforced containment box which can contain any spills that occur during operation or maintenance of the generation system. The containment box is maintained slightly negative to the laboratory atmosphere by the action of the mill's aspirator jet pulling room air into the containment box.

AEROSOL MONITORING

Monitoring of the dye particle mass in the chambers is performed by gravimetric analysis (see Standard Operating Procedure for "Operation of Gravimetric Analysis Equipment for Dye Sampling SOP-J216A-008"). The monitoring system may be separated into three parts: 1) vacuum pumps and manifold, 2) sampling train, and 3) filter probe or cascade impactor.

Vacuum Pumps and Manifold

Figure 7, referred to in previous text, schematically illustrates the vacuum system. Located in a storage shed outside the facility are two vacuum pumps assembled in series and connected to the dye laboratory by copper tubing (1 in.). The vacuum is regulated to 27 in. Hg in a vacuum manifold. The manifold is fitted with four vacuum gauges, stem regulating valves and tubing quick connects.

Sample Train

This system is shown in Figure 14. The sample train contains a dry gas meter (Model DTM-115,, American Dry Gas Meter) with an internally mounted critical orifice for either 2.0 or 28.32 lpm. The dry gas meter is connected to the vacuum manifold via a 12 ft coiled hose and high vacuum solenoid. The solenoid is controlled by a timer for accurate and precise sample collection. The dry gas meter is used to accurately measure the air volume pulled through either the filter probe or the cascade impactor.

Filter Probe or Cascade Impactor

A dry gas meter, with a 28.32 lpm critical orifice is used during sampling with a cascade impactor. Another meter, with a 2 lpm critical orifice, is used for gravimetric sampling using the filter probe. The filter probe is connected to the dry gas meter via a 12 ft coiled hose and a quick connect fitting. The sample probe is fitted with a 25 mm open faced filter holder. The filter holders are positioned through access ports located in the exposure chamber. The cascade impactor (Model 2000, Andersen Samplers, Inc., Atlanta, GA) is attached via a coiled hose (12 ft) and quick connect fitting. Tygon tubing (1 in.) attached to the cascade impactor, enters the chamber through ports in the front or rear of the chamber.

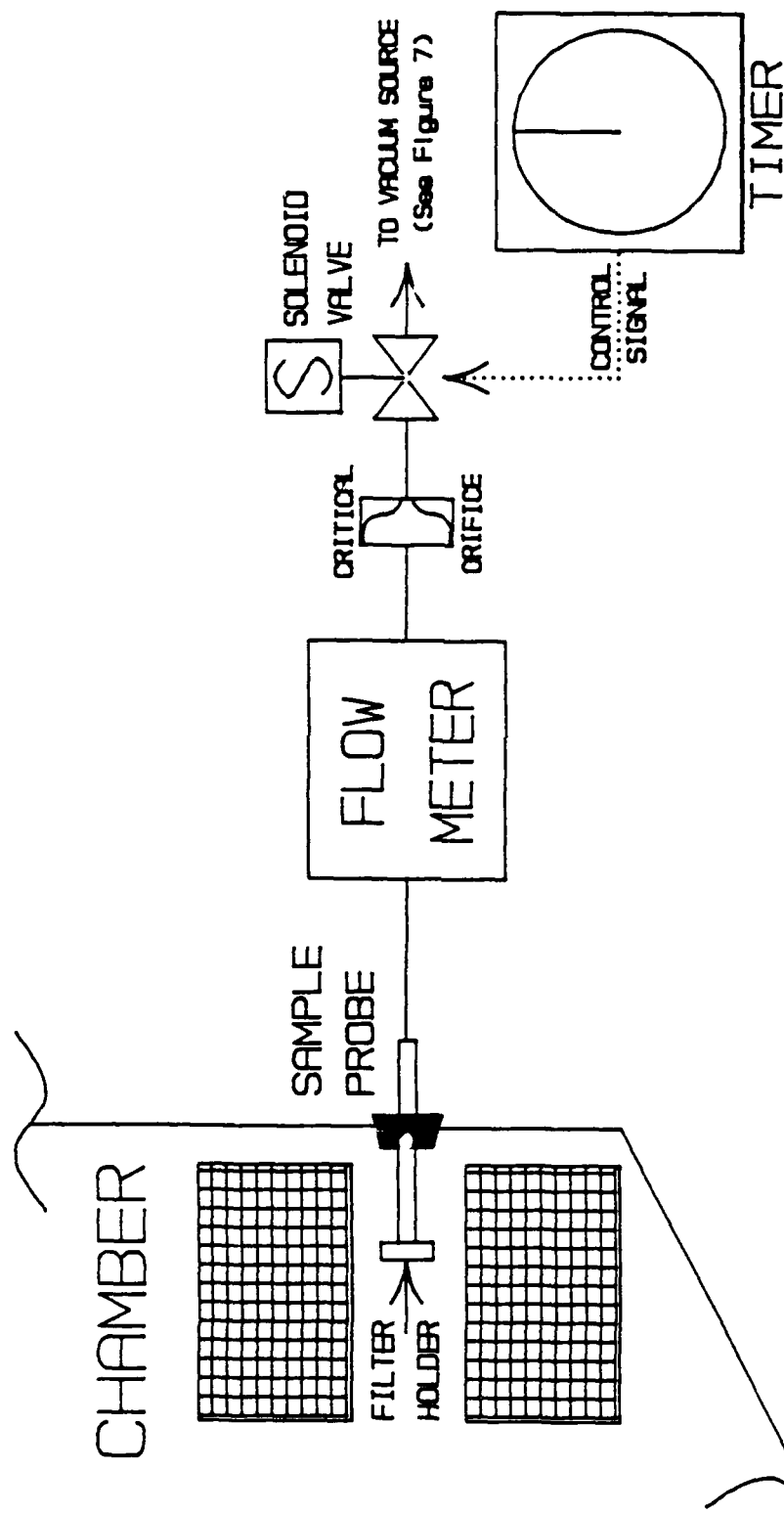


Fig. 14. Aerosol Sampling System

RESULTS AND DISCUSSION

ISOLATION ROOM

The isolated inhalation exposure laboratory was constructed to contain the dye material during operation of the exposure facility. An accidental spill of bulk dye material during a transfer procedure or a spillage of dye contaminated water would create an undesirable condition. It was anticipated that contamination would occur during operation of the exposure facility, and that construction of an isolation room would be needed to prevent spread of the dye material into surrounding areas. Operation of the facility has confirmed that handling the bulk dye and generating aerosols had a high probability of spreading the dye, and, even with care, dye contamination has occurred inside the exposure laboratory. Regular and repeated daily generation of aerosols in the three dye exposure chambers, moving animals, taking aerosol samples for concentration and particle size determinations, and washing chambers and animal cages are procedures that lead to dye contamination of the laboratory. The isolation room has functioned as a reasonable barrier against the spread of the dye into the surrounding laboratories.

AEROSOL CONCENTRATION AND PARTICLE SIZE

A great deal of the initial testing effort was centered on the development of a generation system that would provide a stable aerosol concentration and

particle size for a 6 hr duration. Initial plans were to generate dye aerosol concentrations as high as 1000 mg/m³ with MMADs in the 3-5 μ m range. As testing proceeded, it became clear that to reach this concentration combined with the smaller MMAD size, additional effort and time would be necessary to meet what might, in fact, be levels the toxicologists did not need to investigate.

Previous toxicology data were evaluated and the decision was made to operate the generation system at 200 mg/m³ with an MMAD of less than 3 μ m. Once this criterion was established, work proceeded to obtain these conditions in chamber 4, the prototype chamber. When the concentration and particle size were satisfactory, the additional two exposure chambers were assembled and made ready for operation. Figure 15 illustrates concentrations and MMADs obtained during the facility developmental period up to the date of this report.

It was anticipated that generating particles with the desired MMADs at concentrations around 1000 mg/m³ would be difficult, but it was not anticipated that difficulty would occur with the powder feeder at lower levels. Although initial success was achieved operating the generator up to 6 hr with a relatively stable output, further testing showed that the dye material progressively compacted in the feed tube forcing the helix to slow down and stop. After attempted operation using a plastic-coated and then a teflon nozzle proved unsuccessful, a stainless steel helix (0.375 in.) with stainless steel nozzle was selected. This combination of nozzle, helix, timer and solenoid alleviated most of the helix binding. Once stable operation was achieved over a minimum 6 hr period, aerosol distribution tests were conducted in the prototype chamber.

Mass Median Aerodynamic Diameter

for Red Grenade Mixture

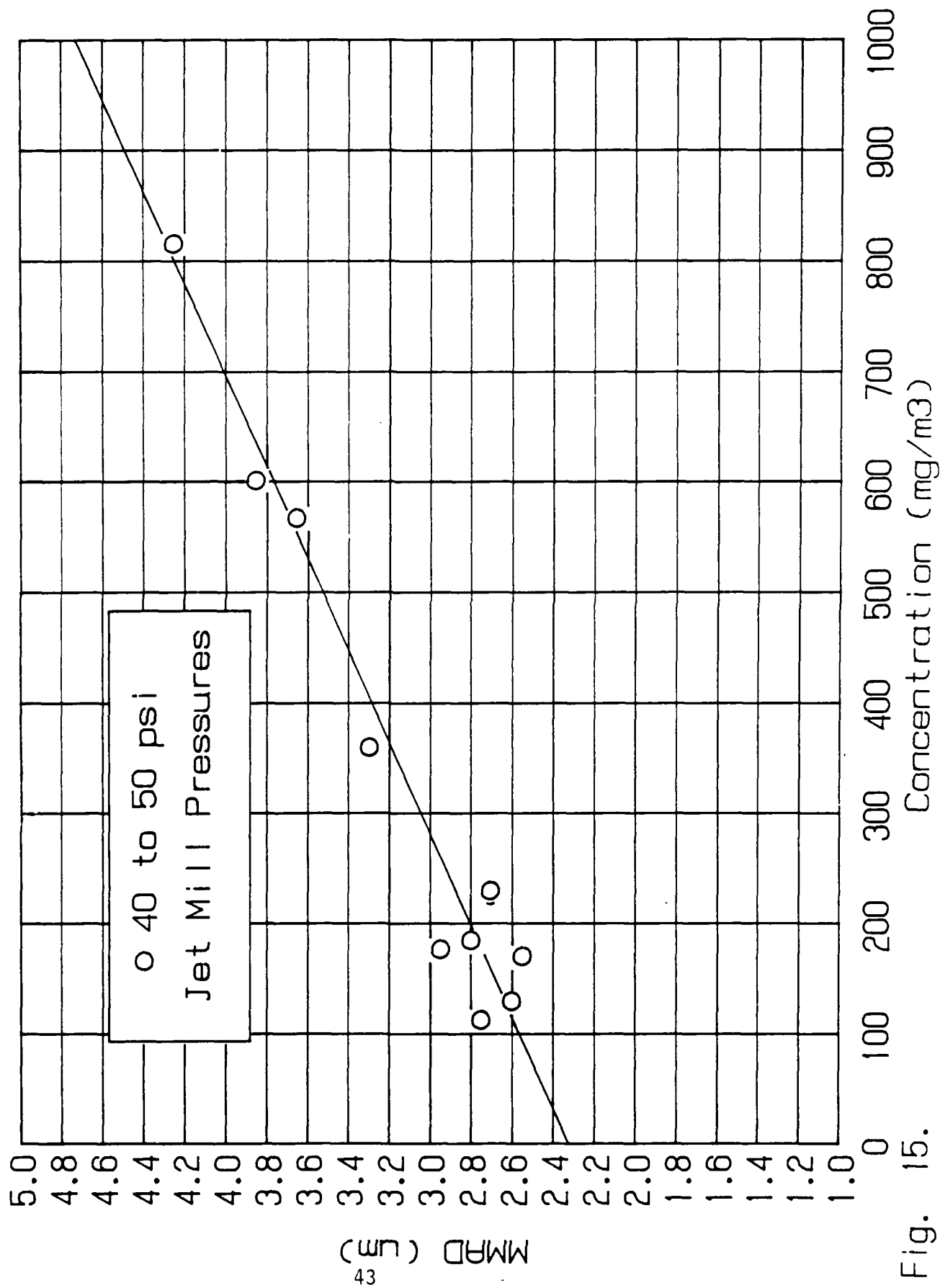


Fig. 15.

The generation system was tested sufficiently to reveal that the smallest particles were generated when the jet mill was operated at the lowest pressure setting. We think this happens because the grinding time in the jet mill is longer at the lower pressure settings than at the higher settings. In order to obtain particle distributions with MMADs of approximately 2.5 μm , the jet mill was operated at 40 psig. For future toxicity testing, it is anticipated the jet mill will be operated at this pressure setting. If it is desired to conduct toxicological testing with particle sizes smaller than 2.5 μm , it is recommended that a properly sized cyclone be installed to remove the larger particles that presently pass the single stage impactor.

AEROSOL CHAMBER DISTRIBUTION

Table C-1 illustrates the results of the distribution study. The MMAD of the dye aerosol was approximately 2.7 μm with a σ_g of 2.25 during the test period. The variation between the grand mean concentrations of filter samples taken at the reference position versus the grand mean concentrations at the sample locations was approximately 5.5%. A previous extensive investigation of the Hazelton 2000 inhalation exposure chamber evaluated the homogeneity of a uranine-CsCl test aerosol with a MMAD of approximately 1.6 μm .⁴ A coefficient of deviation of 8% was noted. Spatial dispersion (as measured in terms of particle mass concentration-mg/m³) of an aerosol with a larger MMAD as used in our work of approximately 3.0 μm , might be expected to be less uniform than that seen with an aerosol having an MMAD of 1.6 μm due to the slightly greater inertia causing increased sedimentation and impaction on chamber structural components. The distribution of the dye aerosol in the

exposure chamber is considered satisfactory for inhalation exposures to evaluate the health effects of the dye aerosol to rodents.

AIR AND WATER FILTRATION

Initial attempts to filter the exhaust air with a wet scrubber were partially successful. The wet scrubber efficiently removed the dye from the chamber exhaust air; however the filtered dye plugged the wet scrubber after about 2 hours of operation. Additionally, the dye-contaminated scrubbing water had to be decontaminated. After further testing, a bag filter was selected, and a housing was fabricated, installed and tested. The inexpensive, replaceable bag filter has proved to filter dye aerosol very efficiently.

Dye contaminated water is generated during operation of the exposure facility. This water, from cage and chamber washing procedures, requires decontamination. A unit developed to remove dye from water effectively decontaminates the water by electrochemical principles. After verification by spectrophotometric analysis that the dye concentration in the water is below 2 ppm, the water is discarded.

FACILITY UTILIZATION

The small-animal inhalation exposure facility described in this report was developed to conduct daily exposures of rodents to dye mixtures that are of interest to the U.S. Army in their colored smoke munitions program. The exposure durations are projected to range from one-time 6 hr exposures to repeated 6 hr subchronic exposures. At the time of preparation of this report, the preliminary design, development and testing of the exposure facility were completed. Based on the results of the distribution tests, plans have been made to proceed with the initiation of the acute animal exposures. If additional modifications are required, documentation of the modifications will be included in the reports dealing with the exposures.

LITERATURE CITED

1. Henderson, R. F., D. E. Bice, Y. S. Cheng, J. S. Dutcher, F. F. Hahn, T. C. Marshall, J. L. Mauderly, M. A. Medinsky, J. A. Pickrell, J. D. Sun, and F. A. Seiler, 1985: Studies on the Inhalation Toxicity of Dyes Present in Colored Smoke Munitions, Final Report for Phase II Studies: Range Finding and Toxicokinetics Studies of Inhaled Dye Aerosols, AD A163267, Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM.
2. Costa, D. L. (1987-1989). Toxicity of Red and Violet Dye Mixtures. Quarterly Reports 1-8. U.S. Environmental Protection Agency, Research Triangle Park, NC. Army Project Order 87PP7808.
3. Moss, O. R., Exposure Chamber. United States Patent 4,216,741. 1980.
4. Beethe, R. L., Wolf, R. K., Griffis, L. C., Hobbs, C. H., and McClellan, R. O. Evaluation of a Recently Designed Multi-tiered Exposure Chamber. Inhalation Toxicology Research Institute, Albuquerque, NM. Final Report 11/79.

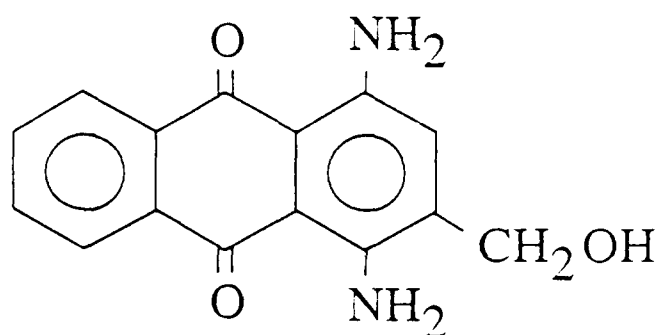
DISTRIBUTION LIST

No. of Copies

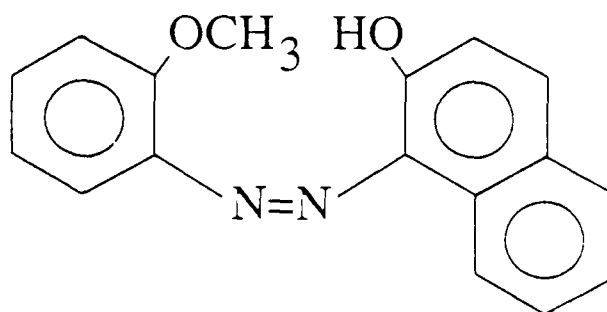
2	Commander US Army Medical Research and Development Command ATTN: CMI-S Fort Detrick Frederick, MD 21701-5012
2	Defense Technical Information Center (DTIC) ATTN: DTIC-DDA Cameron Station Alexandria, VA 22314
1	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20014
1	Commandant Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, TX 78234
30	Commander US Army Biomedical Research and Development Laboratory ATTN: SGRD-UBZ-C Fort Detrick Frederick, MD 21701-5010

APPENDIX A
CHEMICAL STRUCTURES OF DYES

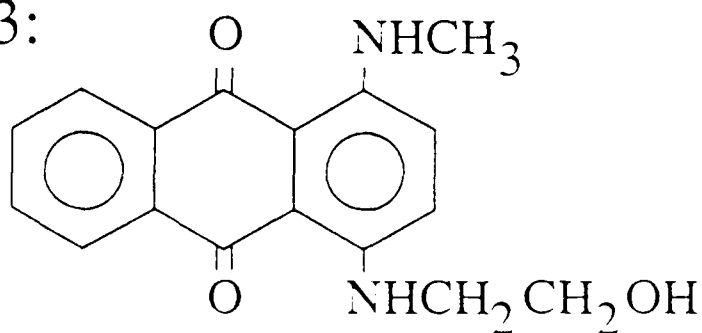
DISPERSE RED 11:



SOLVENT RED 1:



DISPERSE BLUE 3:

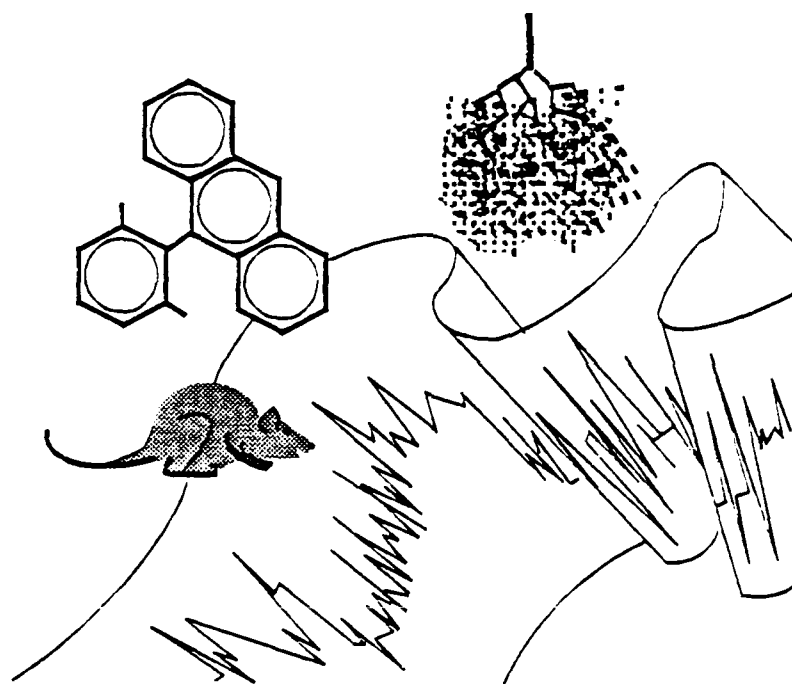


APPENDIX B

STANDARD OPERATING PROCEDURES

STANDARD OPERATING PROCEDURES FOR DYE INHALATION EXPOSURES

SOP-4210-J216A



Submitted to:

U. S. Environmental Protection Agency
Health Effects Research Laboratory
Research Triangle Park, NC 27711

Under Contract No. 68-02-4450

NSI
Environmental
Sciences

STANDARD OPERATING PROCEDURES FOR
DYE INHALATION EXPOSURES

SOP-4210-J216A

for
Health Effects Research Laboratory
U.S. Environmental Protection Agency

This Standard Operating Procedure has been prepared by NSI-ES for the sole use of support personnel in various divisions of the Health Effects Research Laboratory of the U.S. Environmental Protection Agency at Research Triangle Park, NC, and may not be specifically applicable to the activities of other organizations

APPROVALS:

Originator

Mark A. Heginbotham20-JUL-89

Supervisor

L. C. Walsh IIIDate 7/20/89

Taskmaster

David W. DaviesDate 7/20/89

Date

EFFECTIVE DATE:

20-JUL-89

TABLE OF CONTENTS

SECTION	PAGE
SOP J216A-001 Transferring and Handling Dye	54
SOP J216A-002 Calibration of AccuRate Dry Material Feeder for Dyes	56
SOP J216A-003 Calibration of Dye Chamber Orifice Plates	59
SOP J216A-004 Calibration of Temperature Probes	61
SOP J216A-005 Calibration of EG&G Dew Point Hygrometer	64
SOP J216A-006 Critical Flow Orifice Calibration	66
SOP J216A-007 Operation and Calibration of Balance for Gravimetric Analysis	68
SOP J216A-008 Operation of Gravimetric Analysis Equipment for Dye Sampling	70
SOP J216A-009 Operation of Cascade Impactor for Dye Sampling	74
SOP J216A-010 Chamber Distribution for Aerosols	78
SOP J216A-011 Procedure of Start-Up and Operation for Dye Exposure	80
SOP J216A-012 Chamber Sanitation for Dye Exposures	83
SOP J216A-013 Wastewater Color Removal Processes	86
SOP J216A-014 Analysis of Wastewater for the Quantitation of Anthraquinone Dye	88
SOP J216A-015 Procedure for Bag Filter Replacement	91
SOP J216A-016 Procedure for Sampling Chamber Animal Watering System	94
SOP J216A-017 Transporting and Receiving Animals at the Dye Laboratory	98
SOP J216A-018 Procedure for Removing Laboratory Waste	101
SOP J216A-019 Procedure for Ear-Tagging and Weighing Animals	104
SOP J216A-020 Procedure for Weighing Dye Samples	108

SOP J216A-001**1.0 TITLE: Transferring and Handling Dye**

2.0 PURPOSE: To describe the procedure for transferring dye from 55-lb drums to AccuRate[®] hoppers.

3.0 INTRODUCTION

The following SOP may be applied when transferring dye from 55-lb drums to AccuRate[®] hoppers for exposures. The handling of the dye can be hazardous if not performed in a cautious manner.

4.0 SAFETY AND OPERATING PRECAUTIONS**CAUTION**

Harmful levels of these dyes have not been fully determined at this time. Therefore, the dyes should be handled with the utmost care to prevent any potentially hazardous conditions to individuals. The proper safety equipment should always be used by any individuals coming in contact or close proximity with the dyes.

5.0 MATERIALS**5.1 Equipment and Supplies**

- AccuRate[®] Dry Material Feeder model 106
- Utility scoop
- Full-face respirator with particulate cartridge
- Latex gloves
- Whole-body Tyvek[®] suit
- Head cover
- Shoe covers

5.2 Chemical Reagents

- 55-pound drum of violet dye mix
- 55-pound drum of red dye mix

6.0 METHODS

6.1 Remove AccuRate[®] from hood above chamber by removing the electrical connection from the AccuRate[®] controller.

6.2 The person performing the transfer and anyone assisting must wear the following equipment:

- Full-face respirator with particulate cartridge(s)
- Two pairs of latex gloves
- Whole-body Tyvek[®] suit
- Head cover
- Shoe covers

6.3 Place AccuRate[®] feeder on stand.

6.4 Remove 55-pound drum cover and open liner.

6.5 Fill the AccuRate[®] hopper with dye to a safe level without over-filling using the utility scoop stored in drum.

6.6 After transfer is completed, close AccuRate[®] cover, being careful not to spill dye from AccuRate[®] hopper or transfer device.

6.7 Close liner and 55-pound drum cover.

6.8 While handling full AccuRate[®] hopper, make sure cover is in place and do not rest hopper ANYWHERE, but return it to hood above the chamber.

6.9 Reconnect electrical connection to AccuRate[®] controller.

7.0 **DATA PROCESSING:** Not Applicable

8.0 QUALITY CONTROL CHECKS

Analytical analysis of dye drums should be performed initially.

9.0 REFERENCES

9.1 *Operation Manual for AccuRate Dry Material Feeder Model 106.*

SOP J216A-002

1.0 TITLE: Calibration of AccuRate[®] Dry Material Feeder for Dyes

2.0 PURPOSE: To calibrate AccuRate[®] dry material feeder for dye grenade mixtures.

3.0 INTRODUCTION

The AccuRate[®] dry material feeder is used to deliver precise quantities of dye grenade mixtures to Jet-o-mizer[®] grinding mills. These grinding mills reduce particle sizes of the dyes and then deliver the aerosol to the chamber.

4.0 SAFETY AND OPERATING PRECAUTIONS

Refer to SOP "Transferring and Handling Dye" (SOP-4210-J216A-001).

5.0 MATERIALS

5.1 Equipment and Supplies

- AccuRate[®] dry material feeder model 106
- AccuRate[®] controller
- AccuRate[®] hopper extension
- Gravimetric weigh boat
- Mettler semi-micro balance model 163
- Stop watch
- Actuator control box

5.2 Chemical Reagents

- Red grenade mixture and violet grenade mixture

6.0 METHODS

6.1 Set-up of Calibration

- 6.1.1** Calibrate the AccuRate[®] on the cart next to the chamber by removing AccuRate[®] from containment box and reconnecting to the AccuRate[®] controller and actuator.
- 6.1.2** Refer to *Operating and Service Manual of AccuRate Dry Material Feeders* to turn on and for calibration procedures.

- 6.1.3 Make sure feeder has enough material to conduct calibration (at least one-half hopper full).
- 6.1.4 Refer to *Operation Manual of Mettler Semi-micro Balance Model 163* for calibration procedures of balance and set balance to range of 30 g.

6.2 Calibration of AccuRate

- 6.2.1 Set AccuRate[®] controller to 250 (unitless setting) and place weigh boat under nozzle.
- 6.2.2 Adjust actuator speed to 90 cycles/min by turning knob on control box; check rate with stopwatch.
- 6.2.3 Turn on AccuRate[®] and actuator (set to 90 cycles/min) and let run for at least 5 min.
- 6.2.4 Turn off AccuRate[®] and actuator controllers.
- 6.2.5 Start with the lowest AccuRate[®] controller setting and work to the highest calibration point.
- 6.2.6 AccuRate[®] setting for calibration point #1 is:
Controller set = 050
- 6.2.7 Place weigh boat under AccuRate[®] nozzle after taring weight of boat from balance.
- 6.2.8 Turn on AccuRate[®] and actuator and start stop watch simultaneously. After 1 min, stop AccuRate[®] and actuator then weigh sample collected and empty boat.
- 6.2.9 Repeat steps 7 and 8 at least five times at each calibration point setting listed below:
 - AccuRate[®] setting for calibration point #2 is:
Controller set - 100
 - AccuRate[®] setting for calibration point #3 is:
Controller set - 150
 - AccuRate[®] setting for calibration point #4 is:
Controller set - 200
 - AccuRate[®] setting for calibration point #5 is:
Controller set - 250
- 6.2.10 Turn on AccuRate[®] and actuator between each calibration point for at least 2 min at new setting before sampling.

7.0 DATA PROCESSING

- 7.1 Compute powder flow rate in mg/min by:

$$\text{Flow rate} = \text{weight (g) of dye/sample time (min)} \times 1000.$$

- 7.2 Compute mean flow rate (mg/min) for each calibration point.

- 7.3 Plot mean flow ratio (mg/min) vs. AccuRate[®] setting and obtain regression line. This will be used to compute flow rates for specific AccuRate[®] settings.

8.0 QUALITY CONTROL CHECK

This calibration will be performed whenever a QC check point is not within $\pm 10\%$. The QC check point will be performed every time the exposure concentration changes and every six months.

9.0 REFERENCES

- 9.1 Standard Operating Procedure "Transferring and Handling Dye" (SOP-4210-J216A-001).
- 9.2 *Operation and Service Manual for AccuRate Dry Material Feeders.*
- 9.3 *Operation Manual for Mettler Model 163 Balance.*

SOP J216A-003

1.0 TITLE: Calibration of Dye Chamber Orifice Plates

2.0 PURPOSE: To calibrate orifice plates in dye chambers by using Kurz air velocity meter.

3.0 INTRODUCTION

Calibration of the orifice plates in the dye chambers requires the use of the Kurz air velocity meter, since the Roots meter would be contaminated with dye if used.

4.0 Safety and Operating Precautions

Refer to SOP "Transferring and Handling Dye" (SOP-4210-J216A-001).

5.0 MATERIALS

5.1 Equipment and Supplies

- Kurz air velocity and temperature meter model 1440 EPA #880916

5.2 Chemical Reagents: Not applicable

6.0 METHODS

6.1 Refer to SOP "Orifice Plate Calibration" (SOP-4210-J216-005) for calibration procedures.

6.2 Refer to Kurz air velocity and temperature meter model 1440 operation manual for operating procedures.

6.3 Insert Kurz sampling probe into $\frac{1}{4}$ in. hole (covered with duct tape) located on 3 in. PVC pipe before orifice plate but behind HEPA filter.

6.4 Insert 2.75 in. of the sample probe (measured from outside of PVC pipe).

6.5 Rotate probe to achieve highest velocity setting.

6.6 With air flow at zero, set the magnehelic gauge at zero.

6.7 Set first calibration point at 1 in. of H₂O, by opening gate valve.

6.8 Obtain air velocity measurement by switching Kurz meter to 0 to 6000 range and reading first stable air velocity measurement achieved.

6.9 Turn off Kurz meter.

6.10 Adjust next calibration point at 0.8 in. of H₂O, by closing gate valve.

6.11 Repeat steps 8 and 9, for each calibration point remaining at 0.6 in., 0.4 in., 0.2 in., and 0.1 in. of H₂O.

6.12 Repeat steps 7 through 11 in triplicate.

7.0 DATA PROCESSING

7.1 Compute the flow rate (cfm) by:

$$\text{Flow rate (ft}^2\text{/min)} = \text{velocity (ft/min)} \times \text{pipe area (0.045) (ft}^2\text{)}$$

7.2 Compute air flow rate (L/min) by:

$$\text{Flow rate (L/min)} = \text{air flow rate (cfm)} \times 28.32$$

7.3 Compute mean air flow rate (L/min) at each calibration point

7.4 Plot the square of the air flow (L/min) vs. magnehelic setting (inches of water) to obtain regression line.

8.0 QUALITY CONTROL CHECK

The Kurz air flow meter should be audited on an annual basis. The chamber orifice plate calibration should be done every six months.

9.0 REFERENCES

9.1 Standard Operating Procedure "Orifice Plate Calibration" (SOP-4210-J216-005).

9.2 *Kurz Air Velocity and Temperature Meter Operation Manual for Model 1440.*

SOP J216A-004

1.0 TITLE: Calibration of Temperature Sensor Probes

2.0 PURPOSE: To describe the calibration procedure for a LM34 temperature sensor chip for chamber and room monitoring of temperature in degrees F.

3.0 INTRODUCTION

The LM34 temperature sensor chips will be linked to the Molytek portable 32-channel recorder/data logger Model 3702 for continuous real-time temperature monitoring. The temperatures in the chambers and room will be up-dated every 30 s and displayed on a CRT terminal.

4.0 SAFETY AND OPERATING PRECAUTIONS: Not applicable

5.0 MATERIALS

5.1 Equipment and Supplies

- National semiconductor LM34 temperature sensor chips
- Molytek portable 32-channel recorder/data logger Model 3702
- Televideo Model 950 CRT terminal
- Constant temperature bath with stir bar
- Standardized thermometer

5.2 Chemical Reagents: Not applicable

6.0 METHODS

6.1 Refer to *Operating Instruction for Molytek* for Molytek operation, maintenance, and start-up instructions.

6.2 Refer to *Televideo Model 950 User's Guide* for CRT terminal hook-up and operation instructions.

6.3 Remove all temperature probes (containing LM34 temperature sensor chips) from the chambers. Disconnect the electrical connection from those probes and the room probe.

6.4 Relocate probes into constant temperature bath with stir bar and standardized thermometer, located on top of chamber #2.

- 6.5 Start with water temperature of $\approx 84^{\circ}\text{F}$ (standardized thermometer reading), and then span probes by setting ZERO ADJUST on Molytek to 0.
- 6.6 Enter password and "unlock" Molytek, according to operation manual.
- 6.7 Wait for equilibrium and record the voltage reading of each probe by setting TYPE OF CHANNEL to VOLTAGE for channels 1-5 on Molytek. This is calibration point #1.
- 6.8 Decrease temperature to $\approx 80^{\circ}\text{F}$ for calibration point #2 and record all temperature probe readings.
- 6.9 Record all temperature probe readings for each calibration point below
 1. Calibration point #3 – standard temperature $\approx 76^{\circ}\text{F}$
 2. Calibration point #4 – standard temperature $\approx 72^{\circ}\text{F}$
 3. Calibration point #5 – standard temperature $\approx 68^{\circ}\text{F}$
 4. Calibration point #6 – standard temperature $\approx 64^{\circ}\text{F}$
 5. Calibration point #7 – standard temperature $\approx 60^{\circ}\text{F}$
- 6.10 See "Data Processing" (Section 7.0) to obtain regression lines.
- 6.11 Delete old tables (TEMP 1, TEMP 2, TEMP 3, TEMP 4, and TEMP 5) from EXTRA TABLE menu, according to Molytek operation manual.
- 6.12 Add new tables using the same names as before where:
 1. TEMP 1 = Probe channel #1
 2. TEMP 2 = Probe channel #2
 3. TEMP 3 = Probe channel #3
 4. TEMP 4 = Probe channel #4
 5. TEMP 5 = Room probewith the new slope and intercept for each probe.
- 6.13 Set each channel (1-5) under TYPE OF CHANNEL from VOLTAGE to:
 1. Channel 1 – TEMP 1
 2. Channel 2 – TEMP 2
 3. Channel 3 – TEMP 3
 4. Channel 4 – TEMP 4

5. Channel 5 – TEMP 5

according to instructions in operation manual.

6.14 Enter password and "lock" Molytek.

7.0 DATA PROCESSING

Plot voltage (volts) vs. temperature (degrees F) and obtain regression line.

8.0 QUALITY CONTROL CHECKS

The calibration of temperature probes for each chamber and for the room should be performed whenever a calibration check point is not within $\pm 1^\circ\text{F}$. The calibration check point will be performed every three months.

9.0 REFERENCES

9.1 *Operating Instructions, Maintenance, and Trouble-Shooting for Molytek Model 3702 Portable 32 Channel Recorder/Data Logger.*

9.2 *TeleVideo Model 950 CRT Terminal Installation and User's Guide.*

SOP J216A-005

1.0 TITLE: Calibration of EG&G® Dew Point Hygrometer

2.0 PURPOSE: To describe the calibration procedure for a EG&G® dew point hydrometer for measurement of relative humidity in inhalation chambers

3.0 INTRODUCTION

The EG&G® dew point hygrometer is used to measure dew points in inhalation chambers. Dewpoints are then converted to relative humidity readings by using either an Adac computing system or a psychrometric chart.

4.0 SAFETY AND OPERATING PRECAUTIONS: Not applicable

5.0 MATERIALS

5.1 Equipment and Supplies

- EG&G® dew point hygrometer Model 880
- Decade box (for resistance)
- Chart recorder with 0-1 volt range
- Psychrometer

5.2 Chemical Reagents: Not applicable

6.0 METHODS

6.1 Refer to *Instruction Manual for EG&G Dew Point Hygrometer Model 880*.

6.2 Internal hygrometer adjustments that need to be made are CUR LIM, THK, GAIN, and SEN CAL. These are discussed in the instruction manual.

6.3 A chart recorder may be connected to the EG&G® for the THK adjustment, so that the overshoot and undershoot may be better seen.

7.0 DATA PROCESSING

With dry bulb temperature and dew point temperature read from the Molytek, compute relative humidity by using a psychrometric chart. This should closely match the relative humidity on Molytek (from Adac) for each chamber and room.

8.0 QUALITY CONTROL CHECKS

The EG&G[®] calibration should be checked against a psychrometer in a controlled environment.
The relative humidities should be within $\pm 5\%$, and checked every week.

9.0 REFERENCES

- 9.1 *Instruction Manual for EG&G Dew Point Hygrometer Model 880.*

SOP J216A-006

1.0 TITLE: Critical Flow Orifice Calibration

2.0 PURPOSE: To describe the calibration procedure for a critical flow orifice.

3.0 INTRODUCTION

Critical flow is achieved when pressure downstream of the orifice is $\leq 1/2$ of the pressure upstream of the orifice. Realistically, therefore, a maximum flow rate may be obtained that is quite constant. Critical flow orifices are commonly employed on vacuum pumps for air sampling purposes.

4.0 SAFETY AND OPERATING PRECAUTIONS: Not applicable

5.0 MATERIALS

5.1 Equipment and Supplies

- Critical flow orifice of predetermined size
- Flow measuring device (e.g., bubble flow meter, wet test, dry gas meter)
- Stopwatch

5.2 Chemical Reagents: Not applicable

6.0 METHODS

Note: All in-line apparatus to be used experimentally must be placed in-line during the calibration procedure (e.g., flow meters, impingers, filters). Each orifice must be individually calibrated.

6.1 With calibration apparatus in place, adjust vacuum to $< 1/2$ of the atmospheric pressure (~ 16 in. or 406 mm Hg of vacuum at sea level).

6.2 Determine air flow through the orifice by use of a bubble meter or other flow measuring device. Perform this measurement at least three times.

6.3 Ascertain critical pressure by determining air flow through orifice at less and greater vacuum pressure settings.

6.3.1 Adjust vacuum to 1 in. of Hg less than the previously tested pressure (step 1) and determine air flow rates as before (step 2).

6.3.2 Adjust vacuum to 5 in. of Hg greater than the previously tested pressure (step 1) and determine air flow rates as before (step 2).

7.0 DATA PROCESSING

The mean value obtained at each pressure setting must agree to within $\pm 5\%$ to assure achievement of critical pressure.

8.0 QUALITY CONTROL CHECKS

This calibration should be performed whenever calibration apparatus, vacuum, or critical orifice are added or changed. It should also be performed if flow rates differ by more than $\pm 10\%$ from previous orifice calibrations.

9.0 REFERENCES: Not applicable.

SOP J216A-007

1.0 TITLE: Operation and Calibration of Balance for Gravimetric Analysis

2.0 PURPOSE: To describe daily operation and calibration procedures for Mettler semi-micro balance Model 163 for gravimetric analysis

3.0 INTRODUCTION

The Mettler Model 163 semi-micro balance must be checked daily when gravimetric analysis is the monitoring method. This balance requires little maintenance if daily calibration checks are performed.

4.0 SAFETY AND OPERATING PRECAUTIONS: Not applicable

5.0 MATERIALS

5.1 Equipment and Supplies

- Mettler Model 163 semi-micro analytical balance
- Set of class "S" weights (ranging from 1 to 500 mg)

5.2 Chemical Reagents: Not applicable

6.0 METHODS

6.1 Set-Up of Calibration Check

6.1.1 Refer to *Operation Manual for Mettler Model 163 Balance* for start-up and calibration procedures.

6.1.2 Auto-calibrate balance and set upper limit to 30 g, according to operation manual.

6.2 Calibration Check Procedures

6.2.1 Select three weights from set of class "S" weights that are in the range of gravimetric samples to be weighed.

6.2.2 Place each weight individually on the balance and record the accuracy of the balance (Be sure not to touch the weights with your fingers.)

7.0 DATA PROCESSING

Record the balance weights in a notebook for daily comparisons.

8.0 QUALITY CONTROL CHECKS

The calibration checks should be performed every day before weighing begins. When changes are noted from daily recorded weight, the balance may need servicing. The balance should be audited on a yearly basis.

9.0 REFERENCES

- 9.1 *Operation Manual for Mettler Model 163 Semi-Micro Analytical Balance.*

SOP J216A-008

1.0 TITLE: Operation of Gravimetric Analysis Equipment for Dye Sampling

2.0 PURPOSE: To describe the operation of equipment used for gravimetric sampling.

3.0 INTRODUCTION

The equipment used for gravimetric sampling includes a vacuum source, critical orifice, dry gas meter, and sampling probes. This system also has been automated for on/off operation by including a vacuum solenoid and timer. Gravimetric analysis is a simple technique, but requires a high degree of precision.

4.0 SAFETY AND OPERATING PRECAUTIONS: Refer to "Procedure for Transferring and Handling Dye" (SOP-4210-J216A-001).

5.0 MATERIALS

5.1 Equipment and Supplies

- American dry gas meter (1 L/revolution)
- Vacoa high-vacuum solenoid valve
- Fisher timer clock
- 25-mm open-face filter holder and filters
- 0.25-in. stainless steel tubing
- Two 12 ft x 0.25-in. lengths of coiled tubing
- Critical orifice (2 L/min)
- Mettler Model 163 semi-micro analytical balance

5.2 Chemical Reagents: Not applicable

6.0 METHODS

6.1 Set-Up of Gravimetric Analysis

6.1.1 Connect yellow coiled tubing from appropriate dry gas meter (has sampling probes) to coiled tubing from vacuum manifold and open vacuum valve all the way (full vacuum).

6.1.2 Plug timer into wall outlet and ensure that vacuum solenoid is closed by checking movement of dry gas meter (should not be moving).

6.2 Gravimetric Analysis

- 6.2.1 Refer to "Operation and Calibration of Balance for Gravimetric Analysis" (SOP-4210-J216A-007) for proper operation of balance.
- 6.2.2 Remove a glass fiber filter from the desiccator and weigh filter. Note this weight on exposure form (Table 1) under "filter weight (mg)" for proper hour.
- 6.2.3 Load the preweighed filter into the sample probe and insert probe into proper sampling location on the chamber.
- 6.2.4 Record the initial sample volume from the dry gas meter (ex: 345.21) and enter this number on the exposure form under "initial sample volume (liters)" for proper hour.
- 6.2.5 Set sample time to number of minutes and start timer. The total dye weight should not exceed 10 mg or be less than 1.0 mg. The vacuum solenoid should "open" and the dry gas meter should start.
- 6.2.6 Once the timer has stopped, record the "final sample volume" on the exposure form under proper hour.
- 6.2.7 Remove the sample probe and carefully remove the filter from holder and weigh.
- 6.2.8 Record this filter weight on the exposure form under "filter weight and sample (mg)" for proper hour.
- 6.2.9 After weighing, discard the filter into dye material trash.

7.0 DATA PROCESSING

Compute the "sample weight" by subtracting "filter weight" from "filter weight and sample" and record in milligrams on the exposure form. Then compute the "total sample volume" by subtracting "initial sample volume" from "final sample volume" and record in liters on exposure form. Finally, compute analytical concentrations by:

$$\text{analytical conc. (mg/m}^3\text{)} = \text{sample weight (mg)/total sample volume (L)} \times 1000$$

and record under "analytical chamber conc. (mg/m³)" on exposure form.

8.0 QUALITY CONTROL CHECKS

All weights and volumes should be recorded directly on exposure form immediately, so all mistakes can be detected at once by illogical computations. The vacuum system should be checked for leaks from the solenoid to the sample probe on a daily basis by monitoring total volumes on a dry gas meter.

9.0 REFERENCES

- 9.1 Standard Operation Procedure "Operation and Calibration of Balance for Gravimetric Analysis" (SOP-4210-J216A-007).

NSI Technology Services Corporation
Environmental Sciences

Inhalation Exposure Section

Chamber No _____	Sample Time During Flow of _____	Chamber Dry Bulb Temp _____	Chamber Dew Point _____	Chamber Rel Humidity _____	Chamber Air Flow (liters/min) _____	Contaminant Flow Rate (mg/min) or (ml/min) _____	Nominal Chamber Conc (ppm) or (mg/ml) _____	Filter Weight (mg) _____	Filter Weight + Sample (mg) _____	Sample Weight (mg) _____	Initial Sample Volume (liters) _____	Final Sample Volume (liters) _____	Total Sample Volume (liters) _____	Analytical Chamber Conc (ppm) or (mg/ml) _____	% Analytical Ch. Conc. Nominal Ch. Conc. _____	Mass Median Aerodynamic Diameter (Microns) _____	Initials _____
Date _____																	
Day No _____																	
Start Time _____																	
Shut-Down Time _____																	
Total Exp. Hours _____	5.0																
Date _____																	
Day No _____																	
Start Time _____																	
Shut-Down Time _____																	
Total Exp. Hours _____	5.0																
Date _____																	
Day No _____																	
Start Time _____																	
Shut-Down Time _____																	
Total Exp. Hours _____	5.0																
Date _____																	
Day No _____																	
Start Time _____																	
Shut-Down Time _____																	
Total Exp. Hours _____	5.0																
Date _____																	
Day No _____																	
Start Time _____																	
Shut-Down Time _____																	
Total Exp. Hours _____	5.0																

Table 1. Aerosol Monitoring Record

B-1. Aerosol Monitoring Record

SOP J216A-009

1.0 TITLE: Operation of Cascade Impactor for Dye Sampling

2.0 PURPOSE: To describe the operation of the Andersen 2000 Cascade Impactor.

3.0 INTRODUCTION

The Andersen 2000 1 ACFM Particle Fractionating Sampler is a multi-stage, multi-orifice, cascade impactor that is used to measure the size distribution and total concentration levels of all liquid and solid particulate matter. The Andersen nonviable sampler was factory calibrated with unit density (1g/cc) spherical particles so that all particles collected, regardless of their physical size, shape, or density, are sized aerodynamically equivalent to the reference particles.

4.0 SAFETY AND OPERATING PRECAUTIONS: Refer to "Procedure for Transferring and Handling Dye" (SOP-4210-J216A-001)

5.0 MATERIALS

5.1 Equipment and Supplies

- Andersen 2000 cascade impactor
- American dry gas meter (1 L/revolution)
- Vacoa high-vacuum solenoid valve
- Fisher timer clock
- Mettler Model 163 semi-micro analytical balance
- 81-mm glass fiber filter
- Critical orifice (1 cfm)
- Two 12 ft x 0.25-in. lengths of coiled tubing
- 5 ft x 1 in. tygon tubing

5.2 Chemical Reagents: Not applicable

6.0 METHODS

6.1 Set-Up of Sampling

- 6.1.1** Connect yellow coiled tubing from dry gas meter to coiled tubing from vacuum manifold, and open vacuum valve all the way (full vacuum).

- 6.1.2 Plug timer into wall outlet and ensure that vacuum solenoid is closed by checking movement of dry gas meter (should not be moving).

6.2 Particle Size and Distribution Analysis

- 6.2.1 Refer to "Operations and Calibration of Balance for Gravimetric Analysis" (SOP-4210-J216A-007) for proper operation of balance.
- 6.2.2 Refer to *Operating Manual for Anderson 2000 Inc. 1 ACFM Ambient Particle Sizing Samplers* for operating instructions for cascade impactors.
- 6.2.3 Remove 81-mm glass fiber filters from the desicator and weigh a filter. Then load this preweighed filter onto stage 0. Note this weight on Particle Size Distribution Data Form.
- 6.2.4 Weigh a second filter and load preweighed filter onto stage 1. Repeat this procedure until all stages are loaded with filters and all weights are recorded.
- 6.2.5 Connect the cascade impactor 1 in. inlet to the chamber sampling location using the 1-in. tygon tubing.
- 6.2.6 Record the initial sample volume from the dry gas meter (e.g., 345.21) and enter this number on the form.
- 6.2.7 Set sample time to 1 min and start timer. The total individual dye weights on filters should not exceed 10 mg. The vacuum solenoid should "open" and the dry gas meter should start.
- 6.2.8 Once the timer has stopped, record the final sample volume on the form.
- 6.2.9 Remove the 1-in. tygon tubing from chamber and inlet of cascade impactor.
- 6.2.10 Remove each filter one stage at a time (starting with stage 0), and weigh each filter and sample. Record each weight under the proper stage.
- 6.2.11 After weighing, discard each filter into dye material trash.

7.0 DATA PROCESSING

- 7.1 Compute the net weight for each filter by subtracting filter weight from final filter weight. Record in milligrams.
- 7.2 Then, add all the net weights to get total sample weight collected.
- 7.3 Next, divide each net weight by the total sample weight and multiply by 100 to get % weight in size range for each stage.

- 7.4 Finally, compute the cumulative % less than size range by setting stage 8 to "0" and adding each stage together in descending order (e.g., stage 8(%) + 0 = stage 7 cumulative %, while adding stage 7(%) = stage 6 cumulative %, and so on). Follow the format in the Operation Manual for Andersen 2000.
- 7.5 Plot cumulative % less than size range vs. effective cutoff diameter (ECD) (microns) for each stage. These are listed in the operation manual.
- 7.6 Compute the mass median aerodynamic diameter (MMAD) by looking at the particle size with a 50% cumulative % less than the size range.
- 7.7 Compute the particle size geometric standard deviation (micrograms) by:

$$\sigma_g = 84.13\% \text{ diameter} / 50\% \text{ diameter}$$

- 7.8 Compute the net volume by subtracting initial volume (liters) from final volume (liters).
- 7.9 Compute total concentration by dividing total sample weight (milligrams) by net volume (liters) and multiplying by 1000 to get milligrams per cubic meter.

8.0 QUALITY CONTROL CHECKS

The cascade impactor flow rate should be checked on a daily basis by computing net volume divided by the number of minutes. This should be within $\pm 2\%$ of 28.32 L/min (1 cfm). Also, check the condition of O-rings on a daily basis and replace, if needed.

9.0 REFERENCES

- 9.1 Standard Operating Procedure "Operation and Calibration of Balance for Gravimetric Analysis" (SOP-4210-J216A-007).
- 9.2 *Operation Manual for Andersen 2000 Inc. 1 ACFM Ambient Particle Sizing Samplers.*

PARTICLE SIZE DISTRIBUTION DATA

Material:

Initial volume (L) =

Date:

Final volume (L) =

Time:

Principal Investigator:

Net volume (L) =

Chamber no.

Sample time (min) =

Target conc.:

Sample rate (L/min) =

Stage (#)	Tare wt (g)	Final wt (g)	Net wt (mg)	Percent in size range	Cumulative % less than size range	Size range (microns)	ECD (microns)
0						9.0 - 10.0	9.0
1						5.8 - 9.0	5.8
2						4.7 - 5.8	4.7
3						3.3 - 4.7	3.3
4						2.1 - 3.3	2.1
5						1.1 - 2.1	1.1
6						0.7 - 1.1	0.7
7						0.4 - 0.7	0.4
Final						0 - 0.4	0

total net wt =

Analytical concentration (mg/m^3) (total net wt [mg]/net volume [L] $\times 1000$) =

Mass Median Aerodynamic Diameter (MMAD) =

Geometric Standard Deviation (σ_g) =

217-1088

Table B-2. Particle Size Distribution Form.

SOP J216A-010

1.0 TITLE: Chamber Distribution for Aerosols

2.0 PURPOSE: To provide instructions for measuring aerosol test agent distribution within a Hazelton 2000 exposure chamber (2.0-m³ chambers).

3.0 INTRODUCTION

A chamber distribution study is designed to determine uniformity of test atmosphere throughout the inhalation chamber. Quantitative analysis of the test atmosphere will be done with gravimetric analysis. Other analytical methods can be used when gravimetric analysis is inappropriate.

4.0 SAFETY AND OPERATING PRECAUTIONS: Not applicable

5.0 MATERIALS

5.1 Equipment and Supplies

- Mettler Model 163 semi-micro analytical balance
- Sampling manifold
- Vacua high-vacuum solenoid valves
- Fisher timer clock
- Five 25-mm open-face filter holders and filters
- Five critical orifice (1 L/min)
- Real-time aerosol monitor (RAM) S-1
- Hazelton 2000 inhalation exposure chamber

5.2 Chemical Reagents: Not applicable

6.0 METHODS

6.1 Set Up of Distribution Analysis

6.1.1 Calibrate analytical instrument (RAM-1), if used according to operation manual.

6.1.2 Refer to "Operation of Gravimetric Analysis Equipment for Dye Sampling" (SOP-4210-J216A-008) for set-up of gravimetric analysis, and then duplicate the system

6.1.3 Connect sampling manifold to solenoid with timer and load open-face filter holders with filters.

6.1.4 Generate the test atmosphere, allowing enough time for the chamber to reach maximum concentration (T-99)

6.2 Chamber Distribution

6.2.1 Do not change any of the chamber parameters while chamber distribution study is in progress (e.g., chamber air flow or contaminate flow rate).

6.2.2 Connect four sampling probes to desired sampling positions (on the same tier level) in the chamber (at animal breathing zone) and the fifth probe to the reference location in the chamber.

6.2.3 Sample all probes simultaneously by turning on timer clock for the solenoid.

6.2.4 A minimum of the 12 sample ports on the 2.0-m³ chamber should be analyzed to get an accurate chamber distribution. (Each time point will have a reference sample and four sample locations.)

7.0 DATA PROCESSING

Calculate analytical concentration (mg/m³) at desired locations, by using specific flow rate (marked on manifold) for each probe.

The distribution will include at least three time points that contain a total of three reference concentrations and 12 specific locations in the chamber. Statistical design and analysis of data should be handled by a statistician. A student's *T*-test should be used, with a central position in the chamber as the reference point.

8.0 QUALITY CONTROL CHECKS

The instrument required for distribution should be audited on an annual basis.

9.0 REFERENCES

9.1 Standard Operating Procedure "Operation of Gravimetric Analysis Equipment for Dye Sampling" (SOP-4210-J216A-008).

9.2 *Operation Manual for RAM-1 (Real-Time Aerosol Monitoring Device).*

SOP J216A-011

1.0 TITLE: Procedure of Start-Up and Operation for Dye Exposure

2.0 PURPOSE: To provide instructions for initiating daily dye exposure operations.

3.0 INTRODUCTION

The dye generation system uses an AccuRate[®] dry material feeder to deliver dye to a Jet-O-Mizer jet mill for grinding and final delivery of aerosols to the chamber. The system incorporates a single stage impactor for large particle removal and noise reduction systems. The measurement of dye in air is determined gravimetrically using 25-mm, open-face filters.

4.0 SAFETY AND OPERATING PRECAUTIONS: Refer to SOP Table of Contents "Transferring and Handling Dye" (SOP-4210-J216A-001)

5.0 MATERIALS

5.1 Equipment and Supplies

- Jet-O-Mizer grinding jet mill Model 0101
- AccuRate[®] dry material feeder Model 106
- Spencer 2.5-in. OD carbon steel tubing
- EG&G[®] dew point sensor
- Air compressor
- Vacuum pumps
- HEPA filters
- Bag filters
- Manifold fan
- Temperature probes

5.2 Chemical Reagents

- Red and violet grenade mixtures

6.0 METHODS

6.1 Determine whether supply, exhaust, compressor, and vacuum systems are operational by checking appropriate gauges on supply air exhaust air, compressed air, and vacuum manifold.

The supply air manifold should read ≈ 0.5 in. water, exhaust air manifold ≈ 2 in. water, compressed air manifold ≈ 120 psi, and vacuum manifold ≈ 700 mm/Hg.

- 6.2 If any gauge reads below these approximate settings, either go into the storage shed outside and start the compressor or the vacuum pumps, or start the blower fan located on the ceiling of the dye laboratory.
- 6.3 Ensure chamber temperatures are at $70 \pm 2^\circ\text{F}$ for all chambers holding animals as displayed on the CRT terminal.
- 6.4 Ensure all chambers are at least 0.1 in. water negative to room on static pressure gauge
- 6.5 Check each chamber for approximate air flow of 500 L/min.
- 6.6 Load animals into chambers, if they are not already in chambers.
- 6.7 Open compressed air regulator on manifold behind appropriate chamber for exposure. Set regulator 20 psi higher than the needed pressure located on chamber platform next to the dial (e.g., 50 psi is on label next to the dial, so 70 psi is set on the regulator).
- 6.8 Check each pressure gauge on platform to ensure it is set to the appropriate setting marked next to it.
- 6.9 Rebalance chamber air flow (500 L/min) and static pressure (0.1" negative).
- 6.10 Set AccuRate[®] controller feed rate to the appropriate rate for needed chamber concentration.
- 6.11 Turn AccuRate[®] feeder on by pushing AccuRate[®] controller ON switch and actuator on helix by switch on timer box (90 cycles/min).
- 6.12 Note and record start time, AccuRate[®] feed rate, temperature in chambers, and humidity.
- 6.13 Measure and record gravimetric analysis according to "Operation of Gravimetric Analysis Equipment for Dye Sampling" (SOP-4210-J216A-008) on an hourly basis minimum.
- 6.14 Record temperature and relative humidity on an hourly basis (minimum).
- 6.15 Record AccuRate[®] feed rate changes along with time of change.
- 6.16 Vary AccuRate[®] feed rate as necessary to maintain concentrations.
- 6.17 Maintain chamber air flows (≈ 500 L/min) and static pressure (≈ 0.1 and 0.5 in. water) by varying gate valves.
- 6.18 Perform a particle size distribution if this is a single exposure or perform distribution once a week if this exposure is longer than two weeks, according to "Operation of Cascade Impactor for Dye Sampling" (SOP-4210-J216A-009).

6.19 Use the following procedure to stop exposure.

- 6.19.1 Turn AccuRate[®] feeder off by pushing "OFF" switch on AccuRate[®] controller and switch on timer box. Note and record stop time.
- 6.19.2 Compressed air should continue to flow through jet mill for at least 5 min. Then turn off compressed air regulator on manifold.
- 6.19.3 Remove animals from chambers according to SOP "Chamber Sanitation for Dye Exposures" (SOP-4210-J216A-012) after 20 min with an air flow at 500 L/min.

7.0 DATA PROCESSING

Compute mean and standard deviations for daily exposure parameters on exposure form.

8.0 QUALITY CONTROL CHECKS

The following equipment should be checked according to specific calibration SOP for that instrument.

- AccuRate[®] dry material feeders (SOP J216A-002)
- Temperature probes (SOP J216A-004)
- EGG dew point sensor (SOP J216A-005)
- Critical orifice (SOP J216A-006)
- Balance (SOP J216A-007)

The dye drums for exposures should be checked for purity and chemical composition upon delivery.

9.0 REFERENCES

- 9.1 *Operation Manual for Jet-O-Mizer Grinding Jet Mill.*
- 9.2 *Operation Manual for AccuRate[®] Dry Material feeder.*
- 9.3 Standard Operating Procedure "Operation of Cascade Impactor for Dye Sampling" (SOP-4210-J216A-009)
- 9.4 Standard Operating Procedure "Operation of Gravimetric Analysis Equipment for Dye Sampling" (SOP-4210-J216A-008)
- 9.5 Standard Operating Procedure "Chamber Sanitation for Dye Exposures" (SOP-4210-J216A-012)

SOP J216A-012

1.0 TITLE: Chamber Sanitation for Dye Exposures

2.0 PURPOSE: To provide instructions for unloading animals and cleaning up dye exposure chambers.

3.0 INTRODUCTION

The inhalation chambers used for dye exposures will require special procedures for unloading animals and removing dye. These procedures include removing dry material from all surfaces by clean room vacuum cleaner, spray washing chamber, then vacuuming to dry the chamber

4.0 SAFETY AND OPERATING PRECAUTIONS: Refer to SOP "Transferring and Handling Dye" (SOP-4210-J216A-001).

5.0 MATERIALS

5.1 Equipment and Supplies

- Nilfisk clean room vacuum cleaner Model GS80
- Hako wet/dry vacuum cleaner MX-1000
- HydroBlitz spray washer Model 07357
- Dry material vacuum bags for Nilfisk and Hako
- Vacuum attachments
- Catch pan paper

5.2 Chemical Reagents

- NA

6.0 METHODS

Use the following procedures for animal unloading.

- 6.1** Place pre-cut pan paper on floor in front of chamber that will be opened.
- 6.2** Carefully open chamber door so that dye will not spread in exposure room.
- 6.3** Use either Hako or Nilfisk vacuum cleaners to remove dye from chamber doors (make sure Hako vacuum cleaner is set-up for dry material handling, according to operation manual).

- 6.4 Then, carefully vacuum as much dye as possible from the animal cage to be removed. (Be careful not to disturb the animal(s) with the vacuum cleaner.)
- 6.5 Then, carefully slide out animal cage and catch pan together from chamber, so that the animal(s) may be removed from the cage.
- 6.6 Once all animals have been removed, push cage back into chamber and remove the catch pan so paper can be disposed.
- 6.7 Reline catch pan with paper and push catch pan back into chamber.
- 6.8 Continue with next animal cage until all animals have been unloaded from chamber.
- 6.9 Close chamber and dispose of floor paper.
- 6.10 Use the following procedures for chamber sanitation (perform once every two weeks).
 - 6.10.1 Follow above procedures for animal unloading, except do not reline catch pan with paper, just push catch pan back into chamber.
 - 6.10.2 Use the Hako vacuum cleaner set for dry material handling or Nilfisk and attachments to remove as much dye as possible from all surfaces in the chamber and cages.
 - 6.10.3 Set-up HydroBlitz spray washer, as described in operation manual.
 - 6.10.4 Set-up the Hako vacuum cleaner for wet material handling, as described in operation manual.
 - 6.10.5 Place vacuum hose in the bottom of the chamber near the exhaust and turn on vacuum.
 - 6.10.6 Close gate valves on exhaust and supply lines from manifolds to the chamber.
 - 6.10.7 Cover both exhaust and supply air openings in chamber with rubber stoppers.
 - 6.10.8 Carefully spray-wash the inside of the chamber cages and pans with the appropriate disinfectant. (Do not spray up air inlet or exhaust.)
 - 6.10.9 Then use Hako wet vacuum cleaner to dry all chamber surfaces, cages, and pans.
 - 6.10.10 Allow chamber to sit overnight with chamber doors closed before loading chamber with animals.
- 6.11 Disposal of dry and wet materials.
 - 6.11.1 Place all dry material paper bags in double-lined plastic disposal bags and then box and mark for off-site incineration for heavy metal disposal.

6.11.2 Transfer all wet material to 30- or 55-gallon drums and treat for dye removal with Andco system (see operation manual for Andco system and SOP "Wastewater Color Removal Processes" [SOP-4210-J216A-013]).

6.11.3 Dispose of the dye-free water in the sewer system.

6.11.4 Mark the dye sludge for land-fill or off-site incineration for heavy metal disposal.

7.0 DATA PROCESSING: Not applicable

8.0 QUALITY CONTROL CHECKS

The chamber sanitation should be checked by roto-plates before animals are placed into chamber.

9.0 REFERENCES

9.1 Standard Operating Procedure "Transferring and Handling Dye" (SOP-4210-J216A-001).

9.2 Standard Operating Procedure "Wastewater Color Removal Processes" (SOP-4210-J216A-013).

9.3 *Operation Manual for Nilfisk Clean Room Vacuum Cleaner.*

9.4 *Operation Manual for Hako Wet/Dry Vacuum Cleaner Model MX-1000.*

9.5 *Operation Manual for Andco Water Treatment System for Dye Removal.*

9.6 *Operation Manual for HydroBlitz Spray Washer Model 07357.*

SOP J216A-013

1.0 TITLE: Wastewater Color Removal Processes

2.0 PURPOSE: To eliminate high levels of Anthraquinone Dye from the chamber washdown/ sanitization wastewater.

3.0 INTRODUCTION

The "Andco Color Removal Process" is a patent pending electrochemical process that has been designed to reduce the levels of color in the wastewater stream.

4.0 SAFETY AND OPERATING PRECAUTIONS

CAUTION

Heat is evolved when mixing acid. Add the acid to the water slowly and carefully while mixing. Refer to SOP "Transferring and Handling Dye" (SOP-4210-J216A-001).

5.0 MATERIALS

5.1 Equipment and Supplies

- Electrochemical skid containing piping, valves, and the following items.
 - 15-gal acid wash tank
 - 1/3 horsepower process pump
 - electrical enclosure containing switches, pilot light, rectifier, etc.
 - bag filter
 - latex gloves
 - safety glasses
 - laboratory coat
 - Kimwipes

5.2 Chemical Reagents

- Sulfuric acid

6.0 METHODS

6.1 Refer to *Operating Instructions for Andco Color Removal Processes* (Andco Job No. 3-11919)

- 6.2 The wastewater is treated until the concentration of dye is on or below the minimum detectable limit of 2 ppm as measured by the Beckman (Model 25) spectrophotometer.
- 6.3 The processed wastewater (clean) is then disposed of in the building sewer.
- 6.4 Samples of the dirty wastewater before treatment and samples of the processed clean water are taken and saved in the laboratory.
- 6.5 Periodically, the particulate filter is replaced on the Andco unit as specified in the operations manual. The dirty filter bag is placed in double-lined plastic disposal bags and then boxed and marked for off-site incineration or land-fill disposal.
- 7.0 **DATA PROCESSING**
Checks made by the spectrophotometer are logged in a laboratory notebook (see also "Analysis of Wastewater for the Quantitation of Anthraquinone Dye", SOP J216A-014).
- 8.0 **QUALITY CONTROL CHECKS:** Not Applicable
- 9.0 **REFERENCES**
 - 9.1 Standard Operating Procedure "Analysis of Wastewater for the Quantitation of Anthraquinone Dye" (SOP-4210-J216A-014).
 - 9.2 *Operation Manual for Andco Environmental Processes Inc. Color Removal Process.*

SOP J216A-014

1.0 TITLE: Analysis of Wastewater for the Quantitation of Anthraquinone Dye

2.0 PURPOSE: To certify that wastewater from the Dye Aerosol Exposure System has been thoroughly treated prior to disposal in sewage system.

3.0 INTRODUCTION

Visible light spectroscopy is used to quantitate the presence of anthraquinone dye in wastewater. Water is considered clean when dye concentration is <2ppm.

4.0 SAFETY AND OPERATING PRECAUTIONS

CAUTION

Acetonitrile (Methyl Cyanide) is highly toxic. Use in a hood and only when wearing protective clothing.

5.0 MATERIALS

5.1 Equipment and Supplies

- Beckman Model 25 UV/Vis spectrophotometer
- 10-mL pipettes (2/analysis)
- 100-mL erlynmeyer flask
- Matched quartz cuvettes (2)
- Latex gloves
- Safety glasses
- Laboratory coat
- Kimwipes

5.2 Chemical Reagents

- Distilled water
- 100% acetonitrile
- 50% acetonitrile in water
- Dye standards 0.8, 4.0, 8.0, 12.0, 20.0, 40.0 ppm ($\mu\text{g/mL}$ in 50/50 acetonitrile in water)

- Oxford spectro-chek set

6.0 METHODS

6.1 Calibration of spectrophotometer

- 6.1.1 Turn on spectrophotometer 1 h prior to analysis.
- 6.1.2 Obtain treated wastewater aliquot (25 mL) in capped polypropylene bottle. Refer to SOP-J216A-013, "Wastewater Color Removal Processes."
- 6.1.3 Put on protective clothing (glasses, gloves, and laboratory coat).
- 6.1.4 Fill a cuvette with 50/50 acetonitrile and place in the reference cell (R) of the spectrophotometer.
- 6.1.5 Fill a cuvette with 50/50 acetonitrile and place in the sample cell (S) of the spectrophotometer.
- 6.1.6 Set wavelength to 500 nM using the "wavelength adjust" knob.
- 6.1.7 Set the baseline to .000 absorbance units using the "baseline adjust control" knob.
- 6.1.8 Empty contents of sample cuvette, rinse with distilled water, and fill with the 0.8-ppm standard. Clean outside of the cuvette with a Kimwipe.
- 6.1.9 Place in sample cell and record absorbance value.
- 6.1.10 Repeat steps 6.1.8 and 6.1.9 for all standards.
- 6.1.11 Plot absorbance value (X coordinate) vs. concentration (Y coordinate) and perform a linear regression. This is the standard curve for the dye analysis.

6.2 Wastewater analysis

- 6.2.1 Open capped polypropylene bottle in hood and obtain a 10-mL aliquot of treated wastewater. (Make sure sample is thoroughly mixed by shaking bottle.)
- 6.2.2 Transfer aliquot to 100-mL erlynmeyer flask.
- 6.2.3 Add 10 mL of 100% acetonitrile to the wastewater sample in flask. Swirl gently to mix.
- 6.2.4 Fill sample cuvette with wastewater sample and place in sample (S) cell.
- 6.2.5 Record absorbance value.

7.0 DATA PROCESSING

- 7.1 Calculate concentration from the absorbance value using the standard curve.

- 7.2 Multiply concentration by two to obtain actual dye wastewater concentration
- 7.3 Water may be considered clean if concentration < 2 ppm.
- 7.4 if concentration > 2 ppm, water must be run through the ANDCO unit again. See Standard Operating Procedure "Wastewater Color Removal Processes" (SOP J-216A-013).
- 8.0 **QUALITY CONTROL CHECKS**
- 8.1 Check stability of source lamp by using Oxford Spectro-check kit (Fisher Scientific). Refer to kit operating instructions. Perform monthly or after instrument maintenance.
- 9.0 **REFERENCES**
- 9.1 Standard Operating Procedure "Wastewater Color Removal Processes" (SOP-4210-J216A-013).
- 9.2 *Operation Instructions for Oxford Spectro-Check Reagent Kit.*

SOP J216A-015

1.0 TITLE: Procedure for Bag Filter Replacement

2.0 PURPOSE: To provide procedure for bag filter replacement on chambers.

3.0 INTRODUCTION

The exposure chambers in the dye laboratory all have bag filter housings attached. These housings have removable tops for replacing filters when required.

4.0 SAFETY AND OPERATING PRECAUTIONS

Refer to SOP "Transferring and Handling Dye" (SOP-4210-J216A-001)

5.0 MATERIALS

5.1 Equipment and Supplies

- Filtration Technologies bag filter
- Nilfisk or Hako clean room vacuum cleaner
- Full-face respirator with particulate cartridge
- Latex gloves
- Whole-body Tyvek[®] suit
- Safety glasses
- Head cover
- Shoe covers
- Plastic bags
- Incineration boxes

5.2 Chemical Reagents

- NA

6.0 METHODS

6.1 Bag filter removal

- 6.1.1 The person performing the removal and anyone assisting must wear the following equipment.
- Full-face respirator with particulate cartridges
 - Two pair of latex gloves
 - Whole-body Tyvek[®] suit
 - Head cover
 - Shoe covers
- 6.1.2 Close gate valves for exhaust and supply lines to the chamber being serviced. Open chamber doors if animals are being housed in chamber.
- 6.1.3 Carefully open 3-in. quick-connect coupling on bag housing inlet and slide the coupling down.
- 6.1.4 Open the latches (10) which hold the top of housing to the bag filter and carefully remove the top of the box.
- 6.1.5 The top of the box and the top of the bag filter in the box can be vacuumed to minimize contamination of the surrounding area.
- 6.1.6 Carefully remove the bag filter from the housing by lifting the metal band of the bag filter.
- 6.1.7 Once removed, place the bag filter in double-lined plastic bags and then into a cardboard box for off-site incineration.

6.2 Bag filter installation

- 6.2.1 Place a new bag filter in the filter housing making sure the gasket in housing is intact.
- 6.2.2 Place the filter housing cover on the filter box after examining the gasket for breaks.
- 6.2.3 Close the latches on the filter box, noting a positive seal on the bag filter.
- 6.2.4 Reconnect the quick-connect coupling to the filter box inlet.
- 6.2.5 Open the gates valves for both inlet and exhaust lines to the chamber and close chamber doors.
- 6.2.6 Rebalance the chamber airflow for 500 L/min and 0.1 in. negative static.
- 6.2.7 Observe the bag filter seal for leaks.

6.2.8 Place label on filter housing with the date bag filter was changed

7.0 DATA PROCESSING: Not applicable

8.0 QUALITY CONTROL CHECKS

When the pressure drop across the bag filter reaches 0.5 in. of water, the bag filter should be replaced. This is read from the Magnehlic gauge above the filter housing.

9.0 REFERENCES

9.1 Standard Operating Procedure "Transferring and Handling Dye" (SOP-4210-J216A-001).

SOP J216A-016

1.0 TITLE: Procedure for Sampling Chamber Animal Watering System

2.0 PURPOSE: To check chamber animal watering system for quality.

3.0 INTRODUCTION

The water samples taken from chambers in service are checked for fungal, bacterial, and especially pseudomonal infestation to ensure that the animals are receiving clean drinking water. The samples are analyzed by Program Resources, Inc. (PRI), the EPA animal colony contractor.

4.0 SAFETY AND OPERATING PRECAUTIONS

Refer to SOP "Transferring and Handling Dye" (SOP-4210-J216A-001)

5.0 MATERIALS

5.1 Equipment and Supplies

- Nilfisk or Hako clean room vacuum cleaner
- Full-face respirator with particulate cartridge
- Latex gloves
- Whole-body Tyvek® suit
- Safety glasses
- Head cover
- Shoe covers
- Sterilized water sample bottles with lids
- Stick-on labels
- Analysis request forms
- Plastic bags

5.2 Chemical Reagents

- Chlorox/water solution (1 oz/gal), in spray bottle
- Isopropyl alcohol disinfecting solution

6.0 METHODS

6.1 Procedure for water sample collection

NOTE: The sterilized bottles are located in the I-Wing foyer of the Environmental Research Center, on top of the clean area refrigerator.

6.1.1 Label the sterilized, plastic water bottles using stick-on labels (Figure 1).

6.1.2 Complete the analysis request form (Figure 2).

6.1.3 Carefully, open chamber doors to prevent dye laboratory contamination and vacuum any dye from the water outlet of the bottom-most cage battery of the chamber. (When standing in front of chamber, it is the left-hand side.)

6.1.4 Spray this battery water outlet with isopropyl alcohol disinfecting solution.

6.1.5 While wearing dye-free latex gloves, aseptically remove the lid from the water sample bottle. Then, fill the bottle to within 1 in. of the top with water from this same location.

6.1.6 Replace the lid on the water sample bottle.

6.1.7 Repeat these procedures (6.1.1 to 6.1.6) for each chamber in the dye laboratory.

6.1.8 Place the water sample bottles into the designated plastic bag.

6.2 Decontamination procedure

6.2.1 Refer to "Data Processing" for analysis results.

6.2.2 If the analysis report indicates a fungus, bacteria, or especially pseudomonas, then flush watering system on particular chamber with a Chlorox/water solution.

6.2.3 Flush with fresh water until Chlorox is no longer detectable by odor threshold (approximately 5 min of flushing).

7.0 DATA PROCESSING

7.1 Submit samples to PRI by placing them in the I-Wing, clean area refrigerator by 11:30 a.m. Submit analysis request form with samples.

7.2 After the analysis has been returned from PRI, file the completed analysis request form in the Water Sample Results binder located in the dye laboratory.

8.0 QUALITY CONTROL CHECKS

This water analysis procedure should be performed on a weekly basis when animals are being housed for subchronic studies for more than one week.

9.0 REFERENCES

9.1 Standard Operating Procedure "Transferring and Handling Dye" (SOP-4210-J216A-001)

9.2 Standard Operating Procedure "Performing Weekly Water Checks" (NSI-HERL-ITD-TB-ECEL/001)

Position #	_____
Chamber #	_____
Date	_____
Technician	_____

Figure 1. Label for Autoclaved Plastic Water Sample Bottle.

TO:	
FROM:	DATE SUBMITTED:
Type of sample (<i>water, fecal, anal tapes, filter, other</i>) _____	
Test required _____	
Comments _____	

<u>Sample Identification</u>	<u>Test Results</u>
1) _____	_____
2) _____	_____
2) _____	_____
4) _____	_____
5) _____	_____
6) _____	_____
Send a copy of the report to the following people: Elaine Grose, Mail Drop 82 David Davies, Mail Drop 82 Hassel Hilliard, Mail Drop 8	

Figure 2. Analysis Request Form.

Figures B-1. Bacterial Analysis Forms.

SOP J216A-017

1.0 TITLE: Transporting and Receiving Animals at the Dye Laboratory

2.0 PURPOSE: To provide procedure for transporting animals into and out of the dye laboratory

3.0 INTRODUCTION

The dye laboratory is an isolation room to prevent cross-contamination of surrounding laboratories. To maintain this integrity, a pass-through window was installed for the import and export of dye exposure animals. Special filter boxes are also used to eliminate possible contamination once the animals have left the laboratory.

4.0 SAFETY AND OPERATING PRECAUTIONS

Refer to SOP "Transferring and Handling Dye" (SOP-4210-J216A-001)

5.0 MATERIALS

5.1 Equipment and Supplies

- Full-face respirator with particulate cartridge
- Latex gloves
- Whole-body Tyvek® suit
- Head cover
- Shoe covers
- Animal filter shoe boxes
- Nilfisk or Hako clean room vacuum

5.2 Chemical Reagents

- NA

6.0 METHODS

6.1 Procedure for receiving animals into dye laboratory

6.1.1 Meet Principal Investigator at the pass-through window inside dye laboratory.

6.1.2 Allow Principal Investigator to open pass-through on outside of laboratory and load pass-through with animal filter shoe boxes (two boxes wide and two boxes high, for a total of four boxes at any one time).

- 6.1.3 Then allow Principal Investigator to close pass-through window before opening pass-through in the laboratory.
- 6.1.4 Carefully, remove boxes from pass-through and stack on cart, then close pass-through for further animal transport, if necessary.
- 6.2 Procedure for loading animals into chambers
 - 6.2.1 Determine the dose that the animals are to receive and load animals into appropriate chambers.
 - 6.2.2 Place pre-cut pan paper on floor in front of chamber that will be opened.
 - 6.2.3 Carefully open chamber door so that dye will not spread in dye laboratory.
 - 6.2.4 Then, carefully slide animal cage and catch pan out together from the chamber, so that the animals may be loaded into the cage.
 - 6.2.5 Load each animal into individual cage (16/battery) by opening one filter shoe box at a time. Make sure animals have ear tags if this is more than an acute exposure (lasting more than one week).
 - 6.2.6 Once all animals have been loaded, push cage back into chamber.
 - 6.2.7 Continue with next animal cage until all animals have been loaded into chamber.
 - 6.2.8 Close chamber and dispose of floor paper.
- 6.3 Procedure for unloading animals from the chamber
 - 6.3.1 Refer to SOP "Chamber Sanitation for Dye Exposures" (SOP-4210-J216A-012) for procedures for animal unloading.
 - 6.3.2 The exposed animals are placed in the filter shoe boxes in which they were delivered.

CAUTION

Make sure the animals are properly marked as to the dose they received.

- 6.4 Procedure for transporting animals out of dye laboratory
 - 6.4.1 Load animal boxes with filter tops installed from the cart to the pass-through with no more than four boxes.
 - 6.4.2 Close pass-through door and allow Principal Investigator to remove animal boxes by opening pass-through outside of dye laboratory.
 - 6.4.3 Repeat procedure until all animal boxes are transported out of dye laboratory.

7.0 DATA PROCESSING: Not applicable

8.0 QUALITY CONTROL CHECKS

These animals should be properly marked to avoid confusion in animal dosing and ease of rotation.

9.0 REFERENCES

9.1 Standard Operating Procedure "Transferring and Handling Dye" (SOP-4210-J216A-001)

9.2 Standard Operating Procedure "Chamber Sanitation for Dye Exposures" (SOP-4210-J216A-012)

SOP J216A-018

1.0 TITLE: Procedure for Removing Laboratory Waste

2.0 PURPOSE: To provide steps by which to remove waste produced in dye laboratory on a daily basis.

3.0 INTRODUCTION

Dye and animal waste is removed daily from all chambers in service in the dye laboratory. In addition, dye-contaminated clothing is generated at the same rate. This waste is taken to the EPA incinerator for disposal each regular working day.

4.0 SAFETY AND OPERATING PRECAUTIONS

Refer to SOP "Transferring and Handling Dye" (SOP-4210-J216A-001)

5.0 MATERIALS

5.1 Equipment and Supplies

- Full-face respirator with particulate cartridge
- Latex gloves
- Whole-body Tyvek® suit
- Head cover
- Shoe covers
- Heavy-duty plastic bags
- Trash cans (50 and 30 gal)
- Incineration boxes
- Nilfisk or Hako clean room vacuum
- Mop and bucket

5.2 Chemical Reagents

- Ajax cleaner

6.0 METHODS

6.1 Chamber waste removal

- 6.1. The dye-contaminated bag filter is disposed of by following SOP "Procedure for Bag Filter Replacement" (SOP-4210-J216A-015).
- 6.1.2 The dye-contaminated chamber is cleaned by following SOP "Chamber Sanitation for Dye Exposures" (SOP-4210-J216A-012).
- 6.1.3 Remove all soiled (including dye) desorb paper from the chambers and replace with clean stock according to SOP "Chamber Sanitation for Dye Exposures" (SOP-4210-J216A-012).
- 6.1.4 Place papers in plastic bag-lined trash can (30 gal) in dye laboratory. Also insert any other dye waste that is generated, including filter papers and latex gloves.
- 6.1.5 Once the trash can is full, remove plastic bag and seal in second bag and place in cardboard incineration box for on-site incineration.
- 6.1.6 Each regular working day, collect this trash and deliver to EPA incinerator.
- 6.1.7 Clean trash can and insert clean plastic bag.
- 6.2 Dye laboratory waste removal
 - 6.2.1 All contaminated clothing is placed in the 50-gal trash can located in laboratory foyer. This trash can has a plastic liner. Also any other dye-contaminated materials should be placed in this same trash can.
 - 6.2.2 Once the trash can is full, remove plastic bag and seal in second bag and place in cardboard incineration box for on-site incineration.
 - 6.2.3 This trash is removed from laboratory whenever it becomes necessary.
 - 6.2.4 Clean trash can and insert clean plastic bag.
- 6.3 Dye laboratory floor
 - 6.3.1 The dye laboratory floor should be cleaned whenever dye is spilled or when the chambers are cleaned.
 - 6.3.2 The dye-contaminated floor is cleaned with Ajax cleaner, water, a sponge mop, and a bucket.
 - 6.3.3 The dye-contaminated water is stored in floor tanks until transferred to 30-gal drums for dye removal by Andco system. (See SOP "Wastewater Color Removal Processes" [SOP-4210-J216A-013]).

7.0 DATA PROCESSING:

All on-site and off-site incineration requires that request forms accompany the material, listing the material to be incinerated. These forms are available from the EPA incinerator (on site) and the NSI Safety Officer (off site)

8.0 QUALITY CONTROL CHECKS

Refer to SOPs for required quality control checks

9.0 REFERENCES

- 9.1 Standard Operating Procedure "Transferring and Handling Dye" (SOP-4210-J216A-001)
- 9.2 Standard Operating Procedure "Chamber Sanitation for Dye Exposures" (SOP-4210-J216A-012)
- 9.3 Standard Operating Procedure "Wastewater Color Removal Processes" (SOP-4210-J216A-013)
- 9.4 Standard Operating Procedure "Procedure for Bag Filter Replacement" (SOP-4210-J216A-015)

SOP J216A-019

1.0 TITLE: Procedure for Ear-Tagging and Weighing Animals

2.0 PURPOSE: To provide step-by-step instructions for ear-tagging and weighing animals in the dye laboratory when animals are housed in the laboratory for more than two weeks.

3.0 INTRODUCTION

Ear-tagging animals is required for identification and record keeping when animals are housed in chambers for subchronic studies. This provides for an important aspect of animal experiments, which is the accurate determination and permanent recording of animal weights.

4.0 SAFETY AND OPERATING PRECAUTIONS

Refer to SOP "Transferring and Handling Dye" (SOP-4210-J216A-001)

5.0 MATERIALS

5.1 Equipment and Supplies

- Ear tags, with consecutive numbers
- Ear-punching tool
- Animal weight record book
- Individual animal identification cards
- Mettler weighing system Model PK 4800, EPA #333487
- All safety equipment needed to enter laboratory

5.2 Chemical Reagents: Not applicable

6.0 METHODS

6.1 Set up and calibration of balance

NOTE: The balance must have been on for at least 30 min prior to weighing.

6.1.1 Refer to Operation Manual for Mettler Model PK-4800 to calibrate balance.

NOTE: If the application key is in the terminal, remove it before beginning the calibration.

6.1.2 Press the control bar.

6.1.3 Wait for "zero" to appear.

- 6.1.4 Press calibration knob and hold for approximately 1 s.
- 6.1.5 Wait for CAL indication.
- 6.1.6 Place calibration weight on pan and record number.
- 6.1.7 Remove calibration weights.
- 6.1.8 If weight displayed is off by more than 10% of true weight, repeat steps 6.1.2 to 6.1.7.
- 6.1.9 Set the stability detector to the rat symbol and set integration time selector to 5 s.

6.2 Ear-tagging animals

- 6.2.1 Remove animals one at a time from chamber battery cage, sequencing within the chamber, from front to rear and from left to right.
- 6.2.2 Firmly grasp the rat in one hand and then, using the ear-punching tool, clip the ear tag to the right ear of the rat. Make sure that the tag is placed such that the number is on the outside of the ear.
- 6.2.3 Record ear tag number on individual animal identification (ID) card (see Figure 1). Each animal will have an ID card on file throughout the study.

NOTE: Lost or damaged ear tags must be replaced. If an animal's right ear becomes frayed or otherwise damaged, tag the left ear.

6.3 Weighing animals

- 6.3.1 Tare the balance by using a weighing pan.
- 6.3.2 Insert animal statistic application key into terminal with text facing up.
- 6.3.3 Enter the date, time, and first animal number (see page 3 of Mettler Operation Manual).
- 6.3.4 Place the first rat in the weighing pan and press key A.
- 6.3.5 After value has been printed, repeat process with remaining animals, proceeding in numerical order.

7.0 DATA PROCESSING

- 7.1 Within one working day, record printed values in permanent animal weight record book indicating date and study title.
- 7.2 Photocopy the printed tapes from Mettler weighing system.
- 7.3 Store photocopy of printed tapes in the Mettler Printed Tape file binder.

- 7.4 Keep original tapes in dark storage for lifetime of animals.

3.0 QUALITY CONTROL CHECKS

Before weighing animals, check the balance by using a standard set of weights. Indicated values should be within 10% of the standard weight values.

Five animals per chamber are randomly chosen by the Principal Investigator prior to the initiation of study exposures. These animals will be weighed prior to exposure and weekly thereafter on Friday during the daily animal care service. Each animal is identified by an ear tag number.

9.0 REFERENCES

- 9.1 *Operation Manual for Mettler Model PK-4800 Balance.*
- 9.2 Standard Operating Procedure "Transferring and Handling Dye" (SOP-4210-J216A-001).

Principal Investigator _____		
Animal # _____		
Date Received _____	Age _____	Weight _____
Treatment Code _____		Rotation Group _____
Study Investigator _____		
Experiment Number _____		
Date in Chamber _____		Date Out _____
Date Died _____		Technician _____

Figure 1. Animal Identification Card, Form OIEG 04, 1/86.

Figure B-2. Animal Identification Card.

SOP J216A-020

1.0 TITLE: Procedure for Weighing Dye Samples

2.0 PURPOSE: To describe weighing of dye for preparation of stock solutions.

3.0 INTRODUCTION

When stock solutions of various dye mixtures are needed for animal dosing, then dye aliquots are requested by investigators. These dye aliquots are weighed in the dye lab and sealed in vials for delivery.

4.0 SAFETY AND OPERATING PRECAUTIONS

Refer to SOP "Transferring and Handling Dye" (SOP-4210-J216A-001).

5.0 MATERIALS

5.1 Equipment and Supplies

- Mettler model 163 semi-micro analytical balance
- Glass vials, rubber stoppers, and seals
- Weight boats
- Crimping tool
- Spatula

5.2 Chemical Reagents

- Red dye mixture
- Violet dye mixture
- Solvent red I
- Disperse red II
- Disperse blue 3

6.0 METHODS

6.1 Set up and calibration of balance

6.1.1 Refer to "Operation and Calibration of Balance for Gravimetric Analysis" (SOP-4210-J216A-007).

6.1.2 Set upper limit to 100g, according to manual

6.2 Weighing of dye

6.2.1 When dye aliquots are requested, glass vials, rubber stoppers, metal caps, and crimping tools will be delivered to the pass-thru in J216.

6.2.2 The appropriate drum of dye will be opened in the dye holding area and a small amount of dye (enough to fill the number of vials requested) will be removed with a weight boat.

6.2.3 The weight boat will be taken to the balance area and the specified amount of dye will be weighted into each vial.

6.2.4 The specified amount should be marked on each vial and, unless otherwise advised, should be weighted to the nearest tenth of a gram.

6.2.5 When the requested vials of that dye have been filled, the remaining dye should be placed in dry material feeder.

6.2.6 The drum then will be resealed before opening the next drum of dye for aliquots.

6.2.7 This procedure is repeated for each dye (pure or mixture) requested.

6.2.8 Once all vials are filled with the appropriate amount and type of dye, a rubber stopper and metal cap will be assembled on each glass vial.

6.2.9 The sealed vials will be placed in the original carton and placed in pass-thru window for pick up.

7.0 DATA PROCESSING

Record the balance weights in the daily notebook along with type of dye removed.

8.0 QUALITY CONTROL CHECK

The balance should be audited on a yearly basis.

9.0 REFERENCES

Operation Manual for Mettler Model 163 Semi-Micro Analytical Balance.

Standard Operation Procedure "Operation and Calibration of Balance for Gravimetric Analysis" (SOP-4210-J216A-007).

APPENDIX C

PROTOTYPE CHAMBER DISTRIBUTION

WITH

RED DYE MIXTURE

PURPOSE

In the original experimental design, it was determined that the maximum number of rodents to be exposed at any one time would likely be 32. Only two cage modules (one tier) of animals is required for this number of rodents. Therefore, two tiers (four cages) could be eliminated from the exposure chamber minimizing handling of dye contaminated cages. The distribution goal, therefore, was to establish that at least one tier (middle) had homogeneous aerosol concentration ($\pm 15\%$ about the mean).

METHOD AND MATERIALS

Exposure chamber #4 (2 m³ volume) was used as the prototype for systems development and is designed specifically for aerosol inhalation exposures. This chamber has 12 fixed sampling locations into which open face filter holders (25 mm) can be inserted. The distribution test was performed with two sampling probes, one at the geometric center of the chamber, the reference point, and the other at various specific locations in the chamber as shown in Figure C-1. The floating probe when mounted at the various locations placed the filter one foot into the chamber.

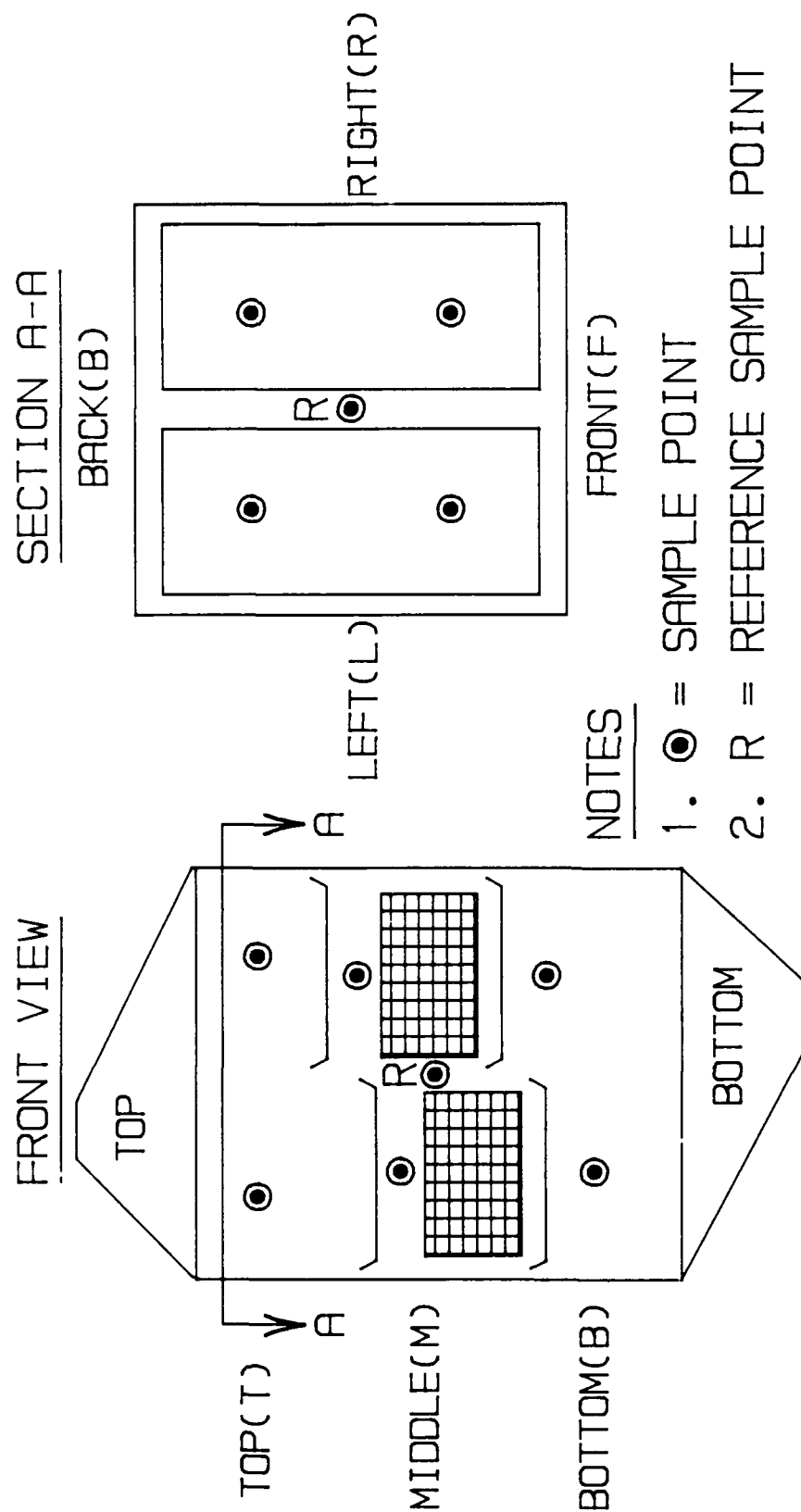


Fig. C-1. Sample Probe Locations For Chamber Distribution Test

The distribution equipment (Fig. C-2) used included a dry gas meter (1 l/revolution) with an internally mounted critical orifice (2 lpm), a high vacuum solenoid, a timer, a stainless steel sampling wand with a 25 mm open face filter holder, and a 12 ft. coiled hose. This system was duplicated so simultaneous sampling could be conducted to eliminate the effect of chamber concentration drift.

Distribution sampling was conducted with the chamber airflow set at 500 lpm and the dry powder feeder (AccuRate) set to supply 350 mg/m³. The chamber was allowed to equilibrate 20 minutes to reach t₉₉ [time required for the chamber concentration to reach 99% of the analytical (actual) concentration]. Filters were placed in both sample probes and inserted into their appropriate positions. The timer was set for 5 minutes and both filter probes collected aerosol samples simultaneously. This procedure was repeated at each sample location listed in Table C-1. The samples were taken 10 minutes apart to evaluate chamber drift and to time-weight each sample. Each location in the chamber (12) was sampled in triplicate.

RESULTS

The variation between the grand means of all filter samples taken at the reference point divided by the grand means of all the floating sample locations was 5.5%. Test results for the middle tier are given in table C-1. Due to the inconsistency of the dry powder feed rate, a technical problem that is presently being addressed, the chamber aerosol concentration drifted during

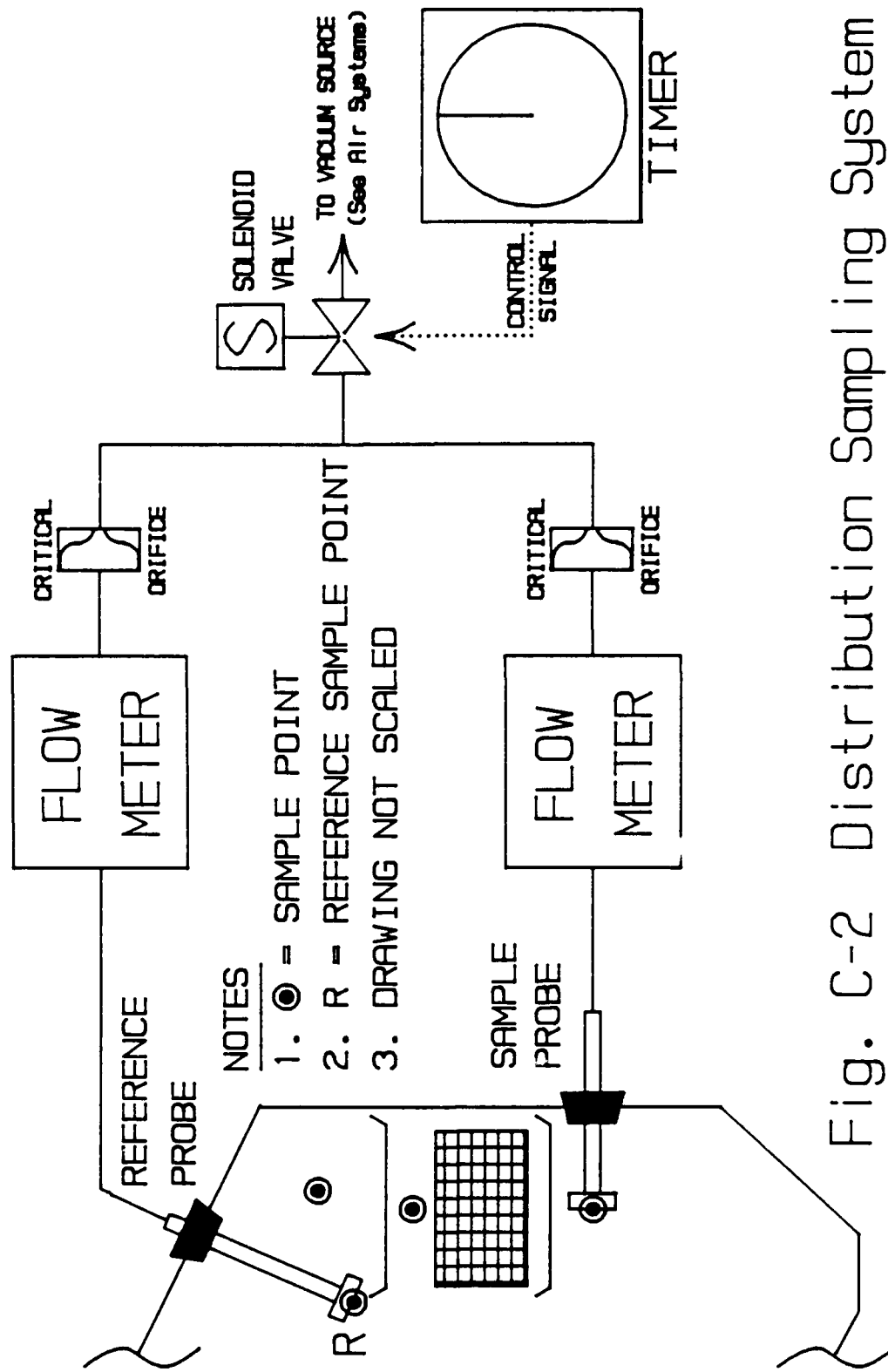


Fig. C-2 Distribution Sampling System

TABLE C-1
DISTRIBUTION DATA

LOCATION	TIME	REFERENCE (MG/M3)	FLOATING (MG/M3)	DIFFERENCE (MG/M3)	PERCENT DIFFERENCE (DIFF/REF)*100
RMF	900	311.69	341.98	30.29	9.72
LMF	910	200.47	215.35	14.88	7.42
RMB	1000	262.91	291.83	28.91	11.00
LMB	1010	278.96	285.36	6.40	2.29
RMF	1100	193.32	210.00	16.68	8.63
LMF	1110	241.25	238.10	-3.15	-1.31
RMB	1200	116.33	126.52	10.19	8.76
LMB	1210	77.56	84.89	7.33	9.45
RMF	1300	203.29	204.69	1.40	0.69
LMF	1310	336.90	306.37	-30.53	-9.06
RMB	1400	152.84	147.83	-5.01	-3.28
LMB	1410	156.65	135.86	-20.79	-13.27

TABLE C-2
SUMMARY STATISTICS
(PERCENT DIFFERENCE)

LOCATION	N	MEAN	STD ERROR OF MEAN	STANDARD DEVIATION
LMB	3	-0.51	6.71	11.62
LMF	3	-0.98	4.76	8.25
RMB	3	5.49	4.43	7.68
RMF	3	6.34	2.85	4.93

the test. The statistical analysis used on the data examined the chamber drift. Statistically, it was found that the chamber drift did not significantly effect distribution analysis. The percent difference between any of the paired floating and reference probes was not greater than 13.3%. Table C-2 shows the means of the percent deviations at each location on the middle tier. There is no more than 7.3% average percent deviation between any two points on this level and 6.3% average percent deviation between any point and the reference probe.

APPENDIX D

NOISE ANALYSIS

Introduction

When the jet mill (Jet-O-Mizer) was selected as the aerosol generator, it was anticipated that noise produced during operation would be excessive. Noise stress on the test animals was considered a problem that should be evaluated prior to initiation of animal studies. This section describes the noise testing conducted in the exposure chambers and in the exposure laboratory.

Materials and Methods

The following equipment was used to conduct the noise level testing:

- Bruel and Kjaer Type 2636 Measuring Amplifier
- Krohn - Hite model 3343 Filter
- Bruel and Kjaer Microphone
- Bruel and Kjaer Calibrator
- Hazelton 2000 Inhalation chambers
- Jet-o-Mizer Grinding Mill model 0101
- Sound insulating foam
- Muffler

All baseline noise measurements were made with animal cages in place and chambers operating at proper airflow. There were two baseline measurements

made for each chamber, one before any modifications and one after. The exposure system tested included an exposure chamber with a jet mill grinder installed, a control chamber (installed in the same room) and a control chamber in another facility (this chamber is designated as the ECEL chamber).

The modifications made to the exposure chamber included removal of one foot of stainless steel tube (3 in.) from the vertical portion of the aerosol inlet to the chamber. This section of tubing was replaced with sound insulating foam. The foam reduced both high and low frequency sound levels. The reduction of room noise was accomplished by attaching a section of sound insulating foam to the aspirator cup of the jet mill.

Fiberglass bag filters were installed downstream of the exposure chambers to filter aerosol from the chamber exhaust. The bag filters acted like mufflers and reduced transmitted noise created by the exhaust blower mounted outside the dye lab. This unintentional reduction of exhaust noise made the control chambers in both locations noisier than the exposure chambers. Therefore, further noise reduction was required in the control chambers. These modifications included installing mufflers in the exhaust lines of both control chambers.

All sound measurements were made with the microphone in the geometric center of the chambers. All sound measurements and calibrations were conducted according to the operations manuals for the equipment used. Each chamber contained a full complement of cages (six) with the catch pans installed.

RESULTS AND DISCUSSION

It was anticipated and demonstrated that the jet mill type of generator would develop high noise levels in the laboratory and in the exposure chambers. The initial noise levels monitored in chamber 3 are shown in Figure D-1. Noise levels are shown with the jet mill generator operated at 40 psi and 60 psi of air pressure. During initial testing, it was determined that the jet mill would be operated in the 40 to 60 psi range. The noise frequency range from 500 Hz to 8 KHz was considered the range that could place stress on the test rodents (personal communication with Raelyn Janssen, Neurotoxicology Division, HERL, EPA) and noise levels greater than 80 decibels were considered to be stressful. The major noise contributor was thought to be noise generated by the jet mill and transmitted into the exposure chamber through the inlet air line. A section of the stainless steel air line was removed and replaced with a section of foam. The effectiveness of the foam liner to reduce noise levels is also shown on Figure D-1 and D-2. The sections of sound insulating foam decrease both laboratory and chamber noise by reducing the amount of reflected noise.

During operation, the jet mill creates a wide frequency range of sound from two supersonic air jets that feed the grinding chamber. This sound is transmitted through the stainless steel tubing into the exposure chamber with little noise level reduction. When this reflected sound comes in contact with the soft foam, the high frequency noise is greatly reduced. While the low frequency noise travels through the soft foam, it is partly absorbed by the

BASELINE JET MILL NOISE TEST

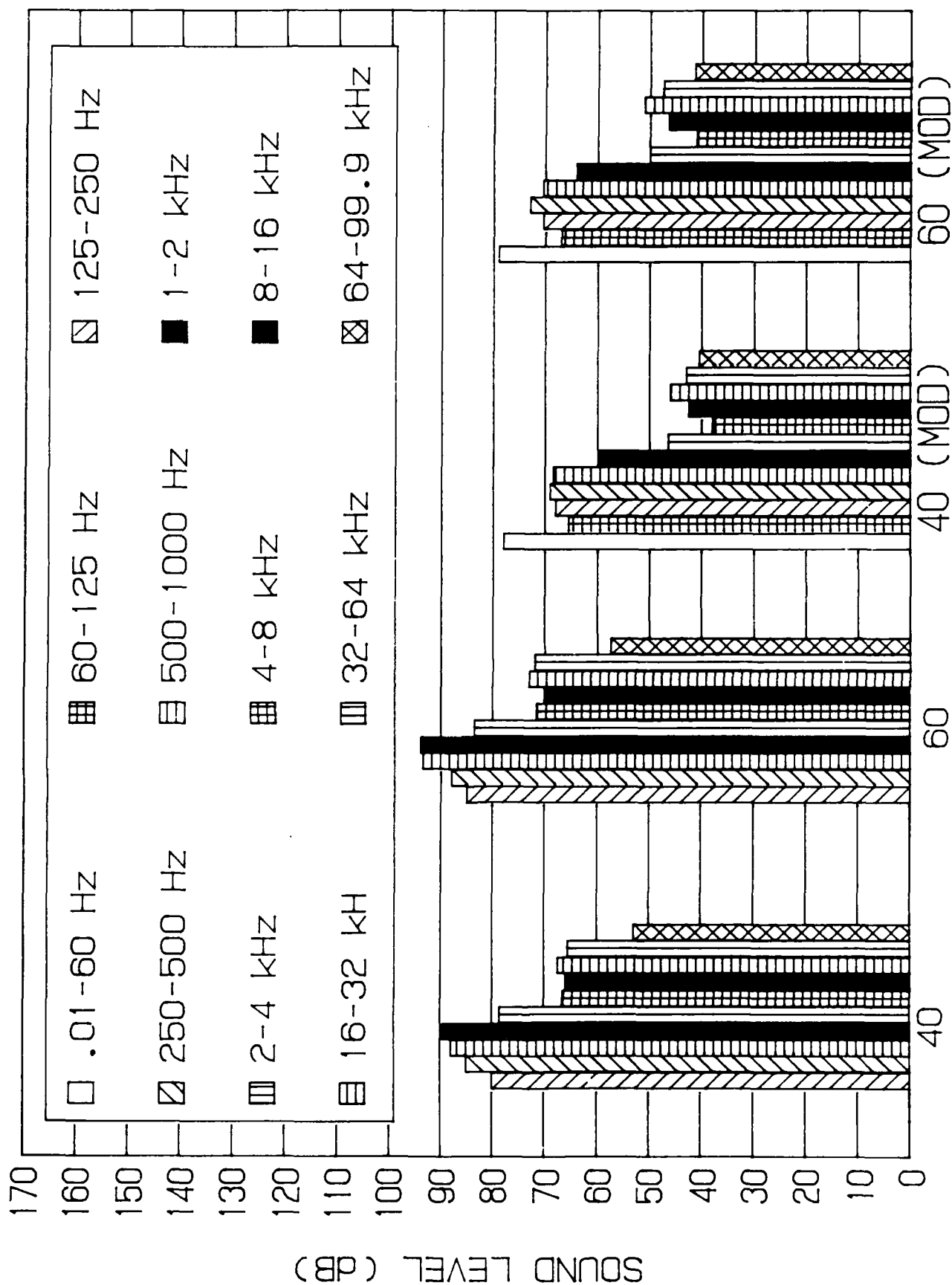


Fig. D-1

CHAMBER 3 (PSI)

JET MILL NOISE TEST

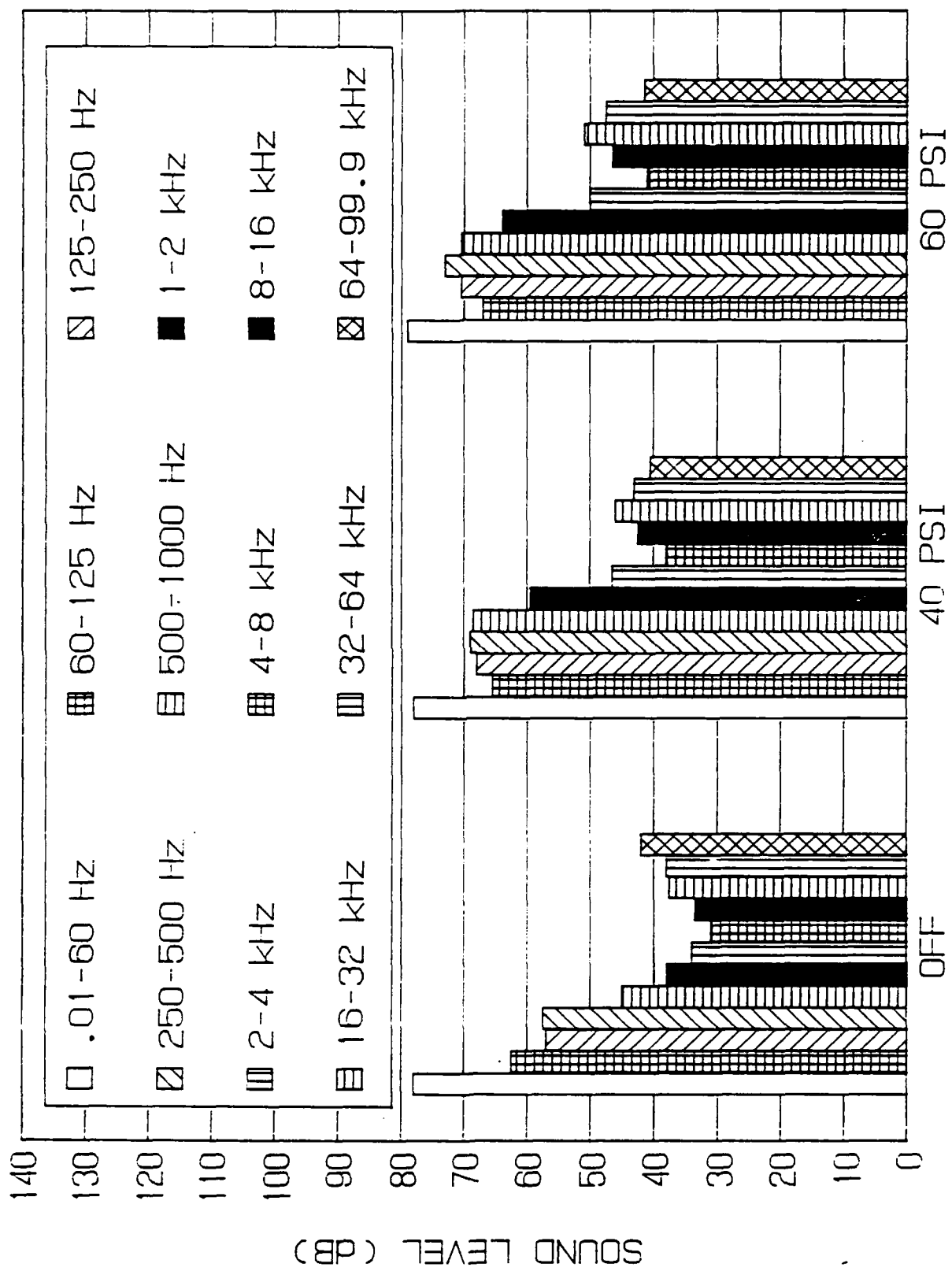


Fig. D-2

CHAMBER 3

harder rubber backing, before reflecting back into the soft foam. The sound insulating foam mounted on the aspirator cup functions as a muffler.

After the noise from the generator was reduced to a suitable level, noise levels were monitored in the control chamber to determine if a "quiet" control chamber and a control chamber with a "dry" jet mill operating should also be operated as a noise control. A "dry" jet mill would operate with the proper air pressure, but without dye feed material.

To determine if it was necessary to operate a standard air control chamber and a "noise" control chamber simultaneously, a test was planned whereby animals would be "exposed" in an air control and a "noise" control chamber for four weeks. Concern was expressed that the jet mill on the exposure chambers might contribute to an unacceptable level of noise in the control chamber, since that chamber was within close proximity to the noise source (operating jet mills). Three exposure chambers were monitored for noise levels during operation. Since chamber 1 in the isolation facility and chamber 2 in the ECEL facility were control chambers, only filtered air would pass through them during operation. Figure D-3 shows the results of noise level monitoring in chamber 3 with the jet mill operating, compared with the noise levels in chamber 1, the control chamber located in the dye laboratory, and a "quiet" control chamber (ECEL) in another laboratory. This baseline noise data shows that, after modifications, the exposure chamber is quieter in the stressful frequency range than both of the control chambers that would be used in the noise study. This finding prompted attempts to reduce the noise in both chamber 1 and ECEL chamber. It was determined that the addition of the large

BASELINE NOISE TEST

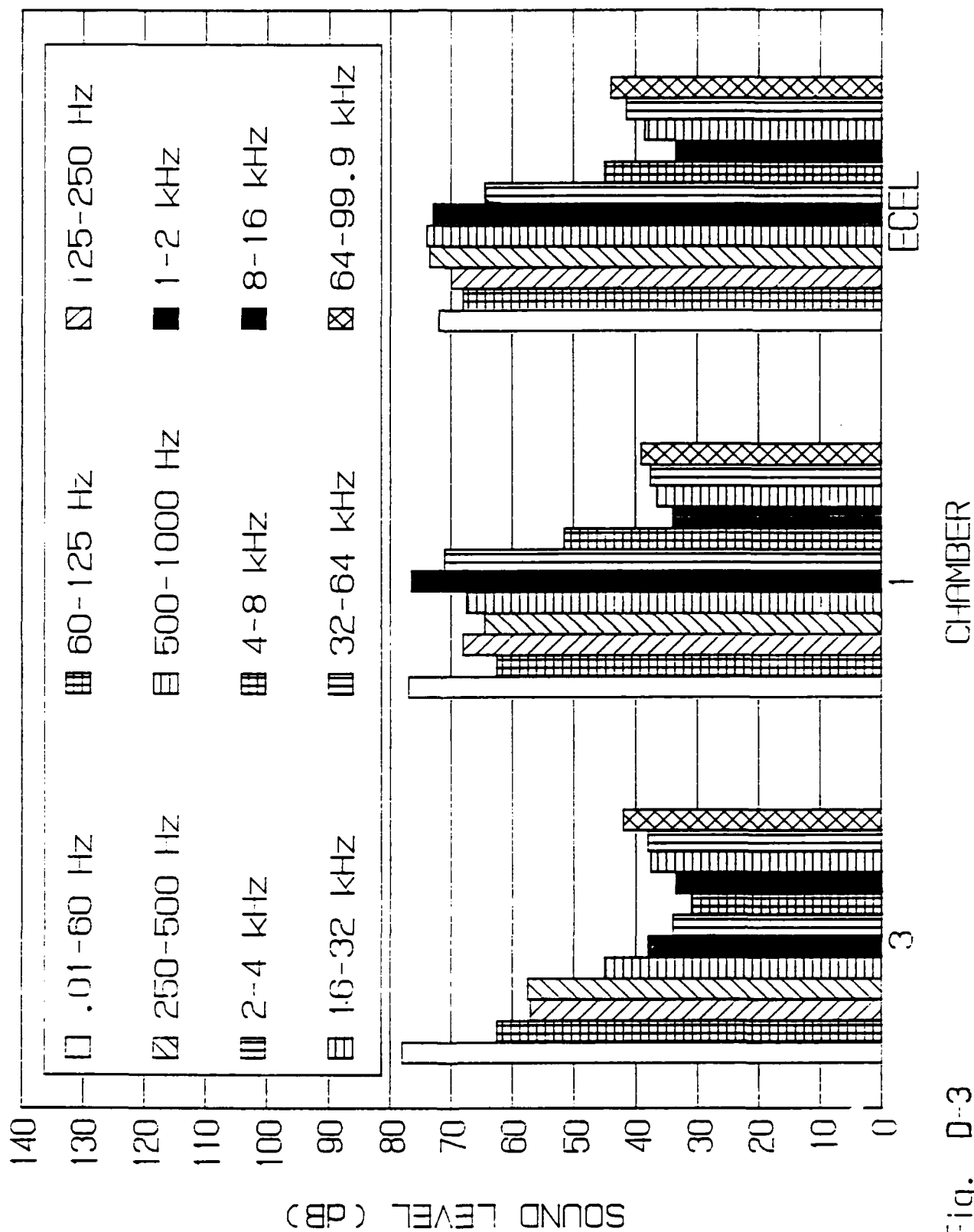


Fig. D-3

bag filter housing, with the bag filter mounted, is an effective noise dampening device. Mufflers were fabricated and installed in the exhaust lines of the control chambers (1 & ECEL), then the noise levels were remonitored. Figure D-4 shows the effectiveness of the mufflers. After the above described work was completed, it was determined that the noise levels in the chambers were at acceptable levels and the proposed noise stress study would not be necessary.

BASELINE NOISE WITH MODIFICATIONS

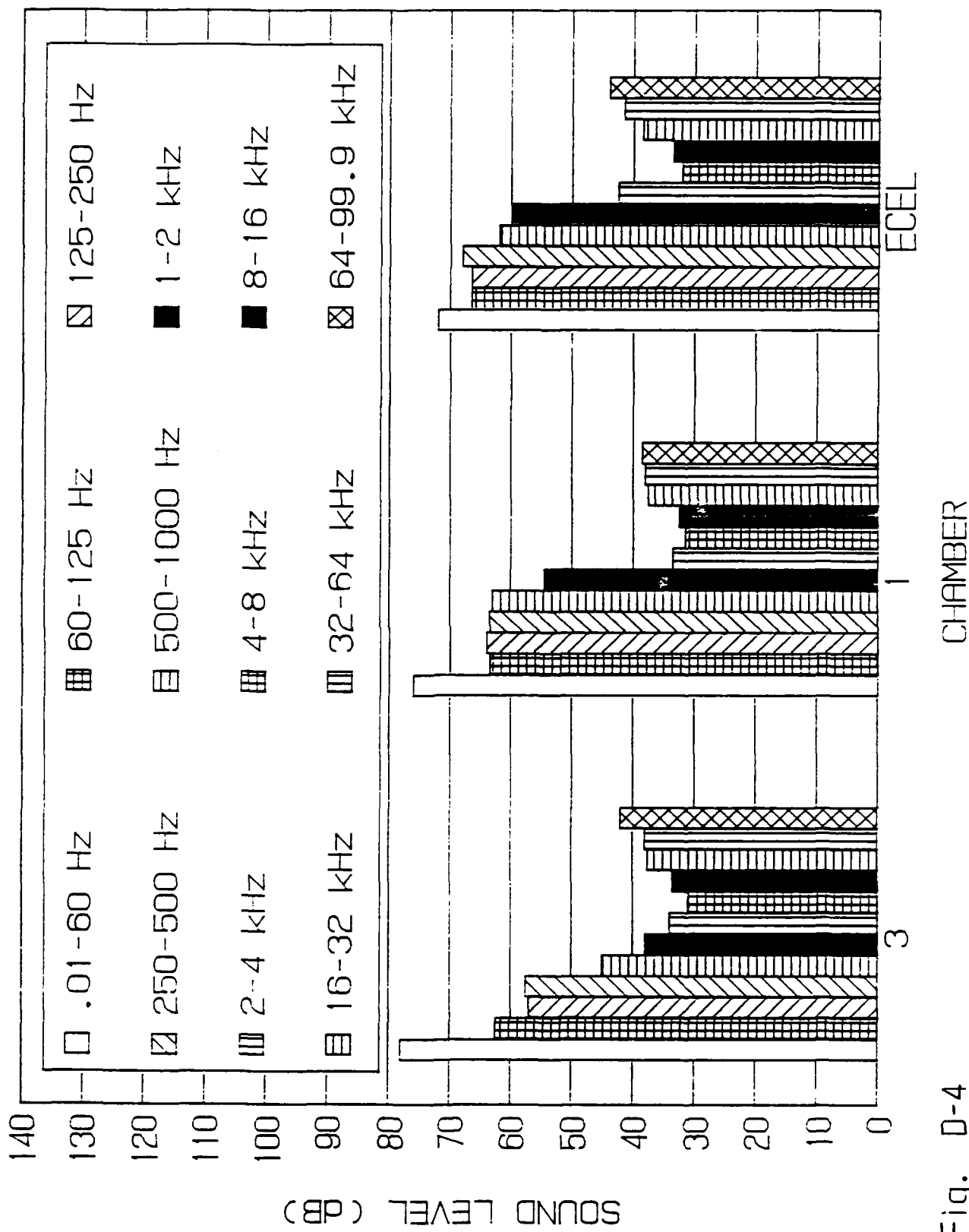


Fig. D-4

APPENDIX E

TABLE E-1. FILTER DATA TABLE

DESIGNATION ON SCHEMATICS	TYPE	FILTER	MANUFACTURER SPECIFICATIONS
A	Bag	Filtration Technology	80-85% of 0.3 μm or greater 95% of 1.0 μm or greater
B	HEPA	Flanders	99.97% of 0.3 μm or greater
C	Vacuum	Wilkerson	5 μm or greater
D	DQ	Balston	93% of 0.1 μm or greater
E	AQ	Balston	99.9999+% of 0.1 μm or greater
Compressed air system	Prefilter	Wilkerson	5 μm or greater
	Coalescing	Wilkerson	99.9999% of 0.03 μm of oil aerosols and solid particles
	Adsorption	Wilkerson	100% of any remaining solid contaminants