PROPOSAL

EVALUATION OF THE ENVIRONMENTAL TOXICANT,
VINYL CHLORIDE MONOMER (VCM)

PROPOSAL NO. B-1648

31 December 1974

For

National Institute of Health
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, North Carolina 27709

Attn: Dr. James S. Woods
Head, Biochemical Toxicology Section
Environmental Toxicology Branch

R&S 131804

MIDWEST RESEARCH INSTITUTE 425 VOLKER BOULEVARD, KANSAS CITY, MISSOURI 64110 • 816 561-0202

R&S 131805

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PREFACE

This proposal, presented to the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, outlines the extension of the work for 12 months from 1 February 1975 through 31 January 1976, and for the succeeding year under the present contract No. NIH-NIEHS-72-2084.

Questions concerning the technical aspects of this proposal should be directed to Dr. W. B. House, Director, Biological Sciences Division. For contractual matters please contact Mr. Robert Donaldson, Contract Administrator. The telephone number of Midwest Research Institute is 816-561-0202. Questions may be directed to the Institute's Washington, D.C., office at 1522 K Street, N.W., telephone 202-293-3800.

R&S 131806

MIDWEST RESEARCH INSTITUTE

Cheng-Chun Lee, Head
Pharmacology and Toxicology

Approved:

W. B. House, Director
Biological Sciences Division

Arthur H. Locke, Manager
Contract Department

TABLE OF CONTENTS

		Page
Introductory Summary		1
I.	Introduction	2
II.	Objectives	3
III.	Experimental Procedure	3
	A. Testing Animals	3
	B. Number, Sex, and Treatment Groups	3
	C. Inhalation Exposures	4
	1. Exposure Chambers	4
	2. Animal Cages	4
	3. Introduction of VCM	4
	4. Analysis and Calibration of VCM in Chambers	5
	5. Holding Chambers	5
	6. Air Handling Systems	5
	7. Exposure Procedure	7
	D. Parameters to be Measured	7
	1. Gross Observation and Growth Rate	8
	2. Hematology and Clinical Blood Chemistry	8
	3. Pulmonary Macrophages	9
	4. Collagen Analysis	10
	5. Immunological Tests	10
	6. Cytogenic Analysis	11
	7. Interim Terminations and Histopathology	11
IV.	Program Organization	12
V.	Level of Efforts and Time Schedule	12
	A. Level of Efforts	12
	B. Time Schedule	13
VI.	Administration and Technical Personnel	13
	A. Administration	13
	B. Technical Personnel	16

R&S 131807

TABLE OF CONTENTS (Concluded)

		Page
	1. Pharmacology and Toxicology.	16
	2. Veterinary Medicine and Pathology.	19
	3. Consultants.	20
	4. Statisticians and Analysts	20
	5. Animal Handlers.	21
VII.	Previous Experience and Current Research Projects.	21
VIII.	Facilities and Equipment	24
	A. General.	24
	B. Pharmacology and Toxicology.	25
	1. Facilities and Equipment	25
	2. Animal Quarters.	25
	C. Facilities and Equipment for the Proposed Studies. . . .	26
IX.	Estimated Cost	28
References.		30

R&S 131808

INTRODUCTORY SUMMARY

Under Contract No. NIH-NIEHS-72-2084, "Long Range Toxicologic Effects of Specific Fungicides," subsequently modified as "Research Capability for Environmental Toxicants," we are investigating the toxic effects of environmental toxicants selected by the Project Officer. During the past 2 years and 10 months, we have studied the commercially available metal-containing dithiocarbamates, including ferbam, maneb, and zinea, and thiram, the degradation product of ferbam. Vinyl chloride monomer (VCM) has been selected for study during the coming contract year.

VCM will be exposed to rats and mice at concentrations of 10, 100, and 1,000 ppm, 6 hours/day and 5 days/week for a minimum of 1 year. The effect of VCM on general behavior will be observed closely and the growth rate will be monitored weekly. Hematology and clinical blood chemistry will be performed at intervals. Pulmonary Type I macrophages will be counted to detect any possible injury to the lung. Collagen in the lung will be assayed and examined for any abnormality. IgA, IgG, and IgM will be quantitated. Cytogenic effects will be studied by chromosomal examination of peripheral lymphocytes. Interim necropsies will be performed to examine any morphological changes in order to correlate with any detectable biochemical changes. Other special tests, when indicated, will be added for the evaluation of VCM effects.

Midwest Research Institute has performed a number of toxicological projects in the past and has a number of current projects in the safety evaluation of a variety of drugs and chemicals. The Program Director (Dr. Cheng-Chun Lee) for the proposed studies, the senior staff (Dr. James V. Dilley, Dr. John R. Hodgson, Dr. Thomas R. Castles, Dr. Chester R. Crawford, Dr. A. Kishan Rao, and Dr. Jaime L. Sanyer), the medical technologists (Mr. T. W. Reddig and Miss J. D. Girvin), the special consultants (Dr. L. D. Kintner in Veterinary Pathology and Dr. H. E. Jensen in Veterinary Ophthalmology), and the supporting staff have extensive experience in various aspects of the proposed studies. The Pharmacology and Toxicology Section of the Biological Sciences Division in which the proposed project will be administered has available animal quarters, laboratory facilities and equipment to perform the proposed studies. In addition, Midwest Research Institute has a number of senior and technical staff who will give advice as well as technical help for the accomplishment of the proposed project whenever the need arises.

R&S 131810

MIDWEST RESEARCH INSTITUTE

PROPOSAL

B-1648

EVALUATION OF THE ENVIRONMENTAL TOXICANT, VINYL CHLORIDE MONOMER (VCM)

I. Introduction

Under Contract No. NIH-NIEHS-72-2084, "Long Range Toxicologic Effects of Specific Fungicides," subsequently modified as "Research Capability for Environmental Toxicants," we investigated the toxic effects of the commercially available metal-containing dithiocarbamates including ferbam, maneb, and zineb, and of thiram, the major degradation product of ferbam. The stability, the metabolism and disposition, the effect on teratology and reproduction, and/or the acute, subacute, and chronic toxicity of these environmental toxicants were studied in rodents.[1-7]

Vinyl chloride monomer (VCM) has been selected by the Project Officer as the next toxicant to be evaluated. VCM was first prepared more than a century ago and its fire and explosion hazard are well known. Acute toxicity of VCM was first reported in guinea pigs.[8] As a possible anesthetic agent in dogs, VCM was found to cause incoordinated muscular activity of the extremities and cardiac arrhythmias[9] and sensitization of the myocardium.[10] Relatively high concentrations of VCM (20-40% in air) for 30 minutes produced narcosis and/or death in mice, rats, and guinea pigs.[11] The guinea pigs were found to be more resistant. The main lesions were congestion of the lung with pulmonary edema and hemorrhages in some animals and congestion of the liver and kidneys. Blood coagulation was also impaired. Various laboratory animals were exposed to 50, 100, 200, or 500 ppm of VCM, 7 hours/day and 5 days/week, for up to 6 months.[12] A threshold limit value of 100 ppm, below which no detectable changes occurred, was suggested. A recent study indicated that rats (Ar/IRE) exposed to high concentration of VCM (3% V/V in air, equal to 30,000 ppm), 4 hours/day, 5 days/week for 12 months, developed tumors of the skin, lungs, and bones.[13] Furthermore, unpublished reports from Italy indicated that liver angiocarcomas were observed in rats exposed to as low as 250 ppm of VCM, 4 hours/day and 5 days/week for 12 months, and from U.S. that mice developed liver angiosarcomas after only 7 months exposure of VCM as low as 50 ppm, 7 hours/day and 5 days/week. The growing suspicion that exposure of VCM has caused a number of deaths of workers from a rare form of liver cancer, angiosarcoma, has alarmed the OSHA to set an emergency rule on April 5, 1974, that lowered the permissible worker exposure level to 50 ppm from the previous level of 500 ppm.

The present proposal outlines the extension of the work for 12 months from February 1, 1975, through January 31, 1976, under Contract No. NIH-HIEHS-72-2084. The chronic toxicity of thiram will be completed, and the complete studies on VCM during the coming contract and the succeeding years are outlined.

R&S 131811

II. Objectives

The specific objectives for the proposed studies are as follows:

1. To determine the biochemical and pathological alterations in specific organ functions of rats and mice chronically exposed to low and mild level doses of VCM.

2. To establish and correlate response profiles of VCM for individual species with clinical data derived from epidemiological studies in humans to be provided by the Project Officer.

3. To evaluate the utility of data derived from chronic toxicity studies of VCM in laboratory animals with regard to its value for predicting toxicity in humans.

III. Experimental Procedure

A. Testing Animals

Young, healthy albino rats (Charles River Breeding Laboratory) and albino mice (National Laboratory Animals) will be employed for the study. The selection of these two species is based on several facts. First, both rats and mice were reported to be sensitive to the toxic effects of VCM.[11] Second, both rats and mice are commonly used laboratory animals. Their hematological and biochemical data are relatively uniform and known. Third, both rats and mice are small in size and large numbers can be used for various parameters of measurement, for interim terminations to extensive morphological changes, and for adequate statistical analysis of results.

B. Number, Sex, and Treatment Groups

A total of 144 males and 144 females of both rats and mice will be used. Each species will be divided into four groups of 36 males and 36 females. Three groups will be exposed to 10, 100, or 1,000 ppm of VCM for 6 hours/day, 5 days/week for a minimum of 1 year. The fourth group will be the control and will be exposed to air under the same conditions as for the treatment groups.

C. Inhalation Exposures

1. Exposure Chambers

The exposure chambers to be used for this study will be the Rochester type manufactured by Young and Bertke, Cincinnati, Ohio. However, a major modification has been made to the door in order to obtain a better seal. This modification replaces the side piano hinge on the door with a top and bottom centerline suspension that allows the door to be closed evenly on the sides, top, and bottom with pressure-type latches. Another modification is that fans will be mounted inside the chambers to insure adequate mixing and distribution of the chamber atmosphere.

Each chamber measures 54 in. x 54 in. x 54 in., not including the top and bottom conical area. This area has four stainless steel wire mesh shelves to support animal cages. The chambers are of stainless steel construction with a large glass window (approximately 48 in. x 48 in.) in the door and two small viewing windows (12 in. x 24 in.) in the rear of the chamber. Sampling ports are located on the side of the chambers to provide access to various locations within the animal exposure area for the purpose of calibrating and monitoring the chamber atmospheres.

R&S 131812

Four chambers will be used for the VCM exposure, one for each level of 10, 100, and 1,000 ppm and a control atmosphere. A fifth chamber will be kept in reserve.

2. Animal Cages

The cages to house the rats and mice during the exposure periods will be the Hoeltge stainless steel (Model HB-34), measuring 9 in. x 15 in. x 8 in. Each cage provides space for two rats or six mice, which is within the space recommendations for these animals based on the Institute of Laboratory Animal Resources Guide (NAS-NRC), 1972. The cages have an open mesh top, sides, and front. Removal of the waste pan leaves an open mesh floor which will provide a minimum impedance to vertical air flow during exposure in the chamber.

3. Introduction of VCM

VCM will be metered from storage tanks through standard corrosion service regulators into stainless steel tubing connected to a calibrated borosilicate flowmeter with a stainless steel float. From the flowmeter the VCM will enter the chamber air supply approximately 12 in. upstream from the top of the chamber. Each chamber will have its own regulator and flowmeter system.

4. Analysis and Calibration of VCM in Chambers

Each chamber will be calibrated for the VCM concentration to be used during the animal exposures. Each of the four shelves in the chamber will be loaded with 12 empty cages and the VCM will be introduced. Samples of the chamber atmosphere will be collected in gas-tight syringes hooked to a polyethylene tube that enters the chamber through a sealed ball-joint port. This arrangement will allow samples to be taken near the four corners and the middle of each shelf. The concentration of VCM will be determined by gas chromatography according to the method described by Sassu et al.[14] Adjustments of the internal fans and the cage positions will be made to insure an essentially uniform VCM concentration in each chamber.

When equilibrium has been established, the settings on the flowmeters and pressure gauges will be noted and replicate samples will be taken from each of the four shelves in the chamber. This process will be repeated with different flowmeter and pressure gauge settings to establish a calibration curve for each chamber which brackets the desired VCM concentration for the animal exposures.

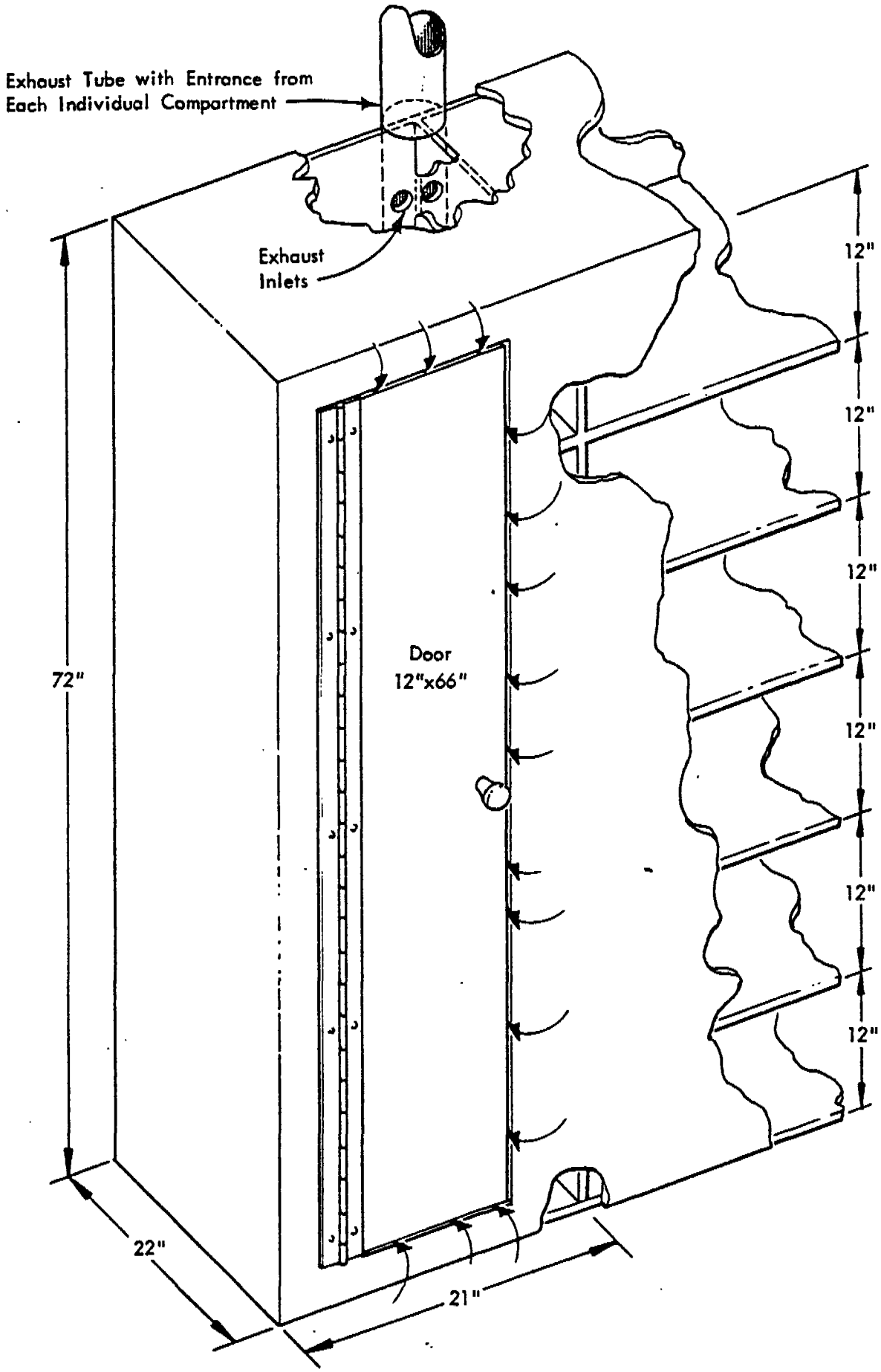
5. Holding Chambers

Lucite holding chambers will be used for storage of animals in their cages during the time when they are not being exposed to VCM. These chambers will be under negative pressure so that room air will be allowed to leak in around the doors, flow over the animals in their cages, and carry any VCM that is off-gassed from the cages, animal pelts, etc., into the exhaust system. Figure 1 shows the detail on one-quarter of a holding chamber. Two animal cages will be kept in each cubicle which measures 21 in. x 22 in. x 12 in. The front door that covers six cubicles will be loosely fitted so that there will be a gap of approximately 3/16 in. on all except the hinged side. Each cubicle is exhausted at the rear through a 1-1/4 in. hole into a verticle 4-in. exhaust pipe. Each exhaust pipe will serve two tiers of cubicles. When a cubicle is not in use, it can be stoppered so that more air flow will be directed through other cubicles that are in use. Only animals from one exposure level will be housed in each holding chamber.

R&S 131813

6. Air Handling Systems

a. Chamber air: Fresh outside air will be drawn through charcoal filters and conditioned prior to entry into the chambers. Air temperature and humidity will be monitored just as it enters the top of each chamber. Exhaust air will be pulled through a chemical filter and then discharged and incinerated through a gas-fired in-line furnace outside the building into a smoke stack. Periodic stack sampling will insure that no vinyl chloride is discharged into the atmosphere.



R&S 131814

Figure 1 - A Diagram of 1/4 of the Lucite Holding Chamber Showing the Dimensions of the Individual Cubicles and the Arrangement of the Exhaust System

Air flow will be regulated at each chamber with a maximum flow rate of 10 chamber volumes/hour. Chamber pressures will be slightly negative to the inhalation laboratory so that no vinyl chloride will escape into the room.

b. Laboratory air: The air for the main laboratory building will be conditioned with a system which is completely separated from the chamber air supply so that no contamination will be introduced into the chamber air supply from the laboratory building.

c. Personnel breathing air: A positive pressure Mine Safety Appliances fresh air mask will be furnished each person working in the inhalation laboratory. These masks will be supplied with fresh compressed air by connecting hoses. These masks will be worn by all personnel while transferring animals in and out of the inhalation chambers, while cleaning the chambers, or whenever a potential inhalation hazard exists.

7. Exposure Procedure

Both rats and mice will be exposed to VCM 6 hours/day and 5 days/week, including holidays. The animals, while still in their cages, will be moved from the holding chamber to the exposure chamber. The feeders and the bottom waste pans will be removed from each cage while it is in the exposure chamber but water will be available to the animals. After each chamber is loaded, the induction of VCM will be started. Samples will be taken from the chamber at designated intervals and analyzed for VCM concentration. When the desired concentration of VCM in the chamber is reached, the exposure time will begin. Each chamber will be started at 10-minute intervals to facilitate sampling from different chambers with a minimum loss of time. During the 6-hour exposure period, each chamber will be sampled every 2 hours to insure a constant concentration of VCM. At the end of the 6 hour exposure period, the VCM will be stopped and the chamber air allowed to flow over the animals for 30 minutes to clear the chamber atmosphere of residual VCM. The animals will then be returned to the holding chambers, the waste pans replaced in the cages, and the animals given measured amounts of food. As animals are removed for periodic sacrifice, their cages will remain in the chamber so that air flow will not be altered.

D. Parameters to be Measured

The following parameters will be carefully examined at the indicated intervals. However, additional tests will be added and the time schedules will be changed if warranted after discussion with and approval by the Project Officer.

1. Gross Observation and Growth Rate

All animals will be observed for behavioral changes and any unusual or toxic signs throughout the study. Body weight of all animals will be recorded weekly at a uniform time of the day. Any unusual changes in weight (loss or gain) will be rechecked immediately. If verified, the underlying cause will be determined. In some cases, the cause may be totally unrelated to the study such as lack of water, excessive food spillage, illness, etc., and proper notation of such will be made.

At the time when animals are being weighed, they will be carefully examined for any apparent deviation from a normal state of health which may have escaped notice during day-to-day observation.

R&S 131816

2. Hematology and Clinical Blood Chemistry

For both rats and mice, various hematologic tests will be performed at the end of 4, 8, 13, 26, 39, and 52 weeks. Four males and four females of the control and all the treated groups will be used. These tests include erythrocyte count, hematocrit, hemoglobin concentration, platelet count, leukocyte and differential counts. In addition, the following clinical blood chemistry tests will be performed for rats and, when possible, also for mice.

a. Serum glutamic-oxaloacetic transaminase (SGOT): SGOT will be measured by the method of Amador and Wacker.[15] Moni-Trol I (Dade), Enza-Trol (Dade), and Reference Serum (Worthington) will be used as the reference for each assay.

b. Serum glutamic-pyruvic transaminase (SGPT): SGPT will be measured by the method of Henry et al.[16] Moni-Trol I, Enza-Trol, and Reference Serum will be used as the reference for each assay.

c. Alkaline phosphatase: Alkaline phosphatase will be measured by the method of Bowers and McComb.[17] Moni-Trol I, Enza-Trol, and Reference Serum will be used as the reference for each assay.

d. Lactate dehydrogenase(LDH): LDH will be measured by the method of Wacker et al.[18] Precinorm E and Precipath E (Boehringer Mannheim Corp.) will be used as the reference for each assay.

e. Bilirubin: Total and direct bilirubin will be measured using the Bili-Strate Kit (General Diagnostics) which is based on a modified Jendrassik-Grof method.[19] Moni-Trol I, Versatol (General Diagnostics), and Calibrate I (General Diagnostics) will be used as the reference for each assay.

f. Total protein: Total protein will be measured by the biuret method of Kingsley.[20] Standard Human Protein (Dade) will be used as the standard for each assay.

g. Albumin: Albumin will be measured by the Albu-Strate Kit (General Diagnostics) which is based on the bromcresol green method of Debro et al.[21] Lab-Trol (Dade) and Calibrate I will be used as the reference for each assay.

h. Globulin: Globulin will be calculated by the difference of total protein and albumin.

i. Prothrombin time: The prothrombin time will be measured by the one-step method of Quick[22] using the Fibro System (BBL) with activated thromboplastin (Dade). Standardized Normal Plasma (Dade) will be used as the control for each assay.

j. BUN: BUN will be measured using the BUN-Strate Kit (General Diagnostics) which is based on the urease method.[23] Three levels of Calibrate (General Diagnostics) will be used to establish a standard curve. Calibrate I, Moni-Trol I, and Versatol will be used as the reference for each assay.

k. Creatinine: Creatinine will be measured by a modified kinetic alkaline picrate procedure.[24] Standard creatinine solutions (Sigma Chemical Company) will be used to establish a standard curve. One level of the standard, Moni-Trol I, and Versatol will be used as the reference for each assay.

3. Pulmonary Macrophages

The pulmonary macrophages are an important defense reaction against foreign substances in the lung. Following the inhalation of different substances, the pulmonary macrophages, Type I cells, respond by both an increase in cell number and phagocytic activity. However, there are conditions such as the presence of a pulmonary infection that have been reported to cause a decrease in the number of Type I cells.[25] The number of cells obtained by pulmonary lavage of rat and mouse lungs after exposure to VCM may be an indicator of the pulmonary damage caused by VCM inhalation.

For both rats and mice, four males and four females exposed to 1,000 ppm of VCM and the control animals will be sacrificed at 4, 8, 13, 26, 39, and 52 weeks. The left lung will be removed at the bifurcation of the trachea, a small needle will be inserted into the bronchus and 5 ml of sterile saline will be gently flushed into the lungs of rats and 0.5 ml in the lungs of mice. The fluid will be withdrawn and flushed in again for 10 times.

R&S 131817

The fluid will be transferred from the syringe to a glass tube and stained. The number of cells in the preparation will be counted under a microscope where a differentiation will be made between macrophages and leukocytes. If changes are observed in the lungs from the animals exposed to 1,000 ppm of VCM, animals from the groups exposed to 100 or 10 ppm will also be examined.

4. Collagen Analysis

Collagen is a structural protein which is involved with repair of injured tissues. In the lung, there is an accumulation of collagen after several types of injury. At the end of 26, 39, and 52 weeks, four male and four female rats exposed to 1,000 ppm VCM and the controls will be exsanguinated and their lungs will be removed immediately for collagen analysis. The tissue will be minced after removing the bronchi and bronchioles and the collagen extracted according to the method of Houck and Jacob.[26] This method depends upon the solubility of protocollagen and collagen in successive extractions of NaCl, citrate, and trichloroacetric acid in the cold. After extraction, each collagen fraction is assayed for total hydroxyproline-proline content as a quantitative measure of the total collagen extracted.[27]

If a difference in the collagen content between the treated and control animals exists, the remaining cold solutions of collagen will be rapidly warmed to room temperature and the collagen fibrils allowed to precipitate. The reconstituted collagen fibrils will be fixed and stained with glutaraldehyde and osmic acid and examined by electron microscopy for abnormal segmented spacing or other indicators of abnormal collagen.

5. Immunological Tests

The immunological surveillance system first proposed by Burnett[28] has gained much support in the last few years. Essentially he has proposed that the immune system provides a natural control of cancer cells that occur frequently, in vivo. A substance which impairs the normal production of antibodies might be expected to enhance the induction of cancer in animals. Therefore, the three major classes of immunoglobins will be assayed during the VCM inhalation exposures.

Aliquots of serum from four male and four female rats exposed to 1,000 ppm VCM and from control rats sacrificed at 4, 8, 13, 26, 39, and 52 weeks will be assayed for immunoglobin content (IgA, IgG, and IgM). The radial immunodiffusion technique described by Mancini et al.[29] will be used for the assay. A replicate serum sample from each rat is placed in wells in an immunodiffusion chamber along with a standard and held until the precipitin ring has reached equilibrium. The square root of the diameter of the precipitin ring is directly proportional to the concentration of antibody.

R&S 131818

Therefore, this method will detect any early quantitative change in serum antibody content.

6. Cytogenic Analysis

Examination for any alterations of the lymphocyte chromosomes will be performed. Peripheral blood samples will be collected from four males and four females of the control group and the group exposed to the highest concentration of VCM (1,000 ppm) at the end of 4, 8, 13, 26, 39, and 52 weeks. Blood samples from animals exposed to 10 or 100 ppm of VCM will also be collected and examined if warranted.

a. Preparation of cultures: Lymphocyte cultures will be made according to the procedure of Moorhead et al.[30] All cultures will be maintained in Ham's Nutrient Mixture F12 supplemented with 15% fetal calf serum.[31]

b. Chromosome analysis: Actively dividing cultures (phytohemagglutin stimulated) will be arrested in metaphase by subjecting them to short-term colchicine treatment. The cells will be collected, swollen in hypotonic saline and processed for spreading on glass slides according to the method of Moorhead and Nowell.[32] Slides will then be stained with giemsa and scanned under low power optics. Those slides showing a minimum scattering of cells in metaphase will be selected for chromosomal analysis using oil immersion optics. Estimation of cell polyploidy level will be obtained by rough chromosomal estimates of at least 200 cells. Exact chromosome counts and chromosomal aberration estimates will be determined from at least 50 metaphase cells. We will be mainly concerned with chromosomal damage such as breaks, gaps, exchanges and dicentrics. Karyotype analysis will be done when warranted. Chromosomes will be classified according to the total length and position of the centromere on each chromosome.

7. Interim Terminations and Histopathology

Four males and four females from the control group and the three treated groups will be euthanatizad for necropsy at the end of 4, 8, 13, 26, 39, and 52 weeks. Gross examination of all tissues, especially for the appearance of abnormal growth, will be carefully performed. The salivary glands, brain, lungs, liver, gall bladder, both kidneys, both ureters, urinary bladder, spleen, mesentery lymph node, and any abnormal tissues will be removed, fixed, sectioned, and stained for microscopic examination. The bones of the extremities will be saved for X-ray scanning of any appearance of oesteolysis.

R&S 131819

Staff	Man-Months
Principal Investigator	3
Senior Toxicologist	6
Associate Biologist	6
Associate Pathologist	1.5
Medical Technologist	2
Junior Biologist	18
Laboratory Assistant	10
Animal Handler	12
Consultants Computer Operators	1
Total	59.5

B. Time Schedule

Time schedule for performing the various proposed studies during the 4th and the 5th contract years is shown in Figure 2. However, the time schedule for the various studies is flexible. Certain studies may be deleted and special studies may be added when warranted and approved by the Project Officer.

VI. Administration and Technical Personnel

A. Administration

Midwest Research Institute is organized into four divisions (Figure 3). Each project at the Institute is administered by one of these divisions; however, personnel from different divisions routinely work on projects requiring the skills of more than one discipline. The Biological Sciences Division consists of four sections: Ecological Assessment, Life Sciences, Medicinal Chemistry, and Pharmacology and Toxicology.

F. V. Morriss, Vice President, Technical Operations: At MRI since early 1953, Dr. Morriss was previously Head of the Organic Chemistry Section for 7 years, and Director of the Chemistry Division for 4 years. As Section Head, he had technical and administrative responsibility of a group performing organic chemical synthesis and air pollution studies. As director, he administered a staff of over 75 analytical, organic, physical, and inorganic chemists, and chemical engineers performing research and development in widely diversified areas of chemistry and chemical engineering. As Vice President, his responsibility is the overall management of MRI's technical operations, including the planning, organizing and coordinating of all research and professional activities in areas of biological sciences, chemistry, engineering, and mathematics and physics.

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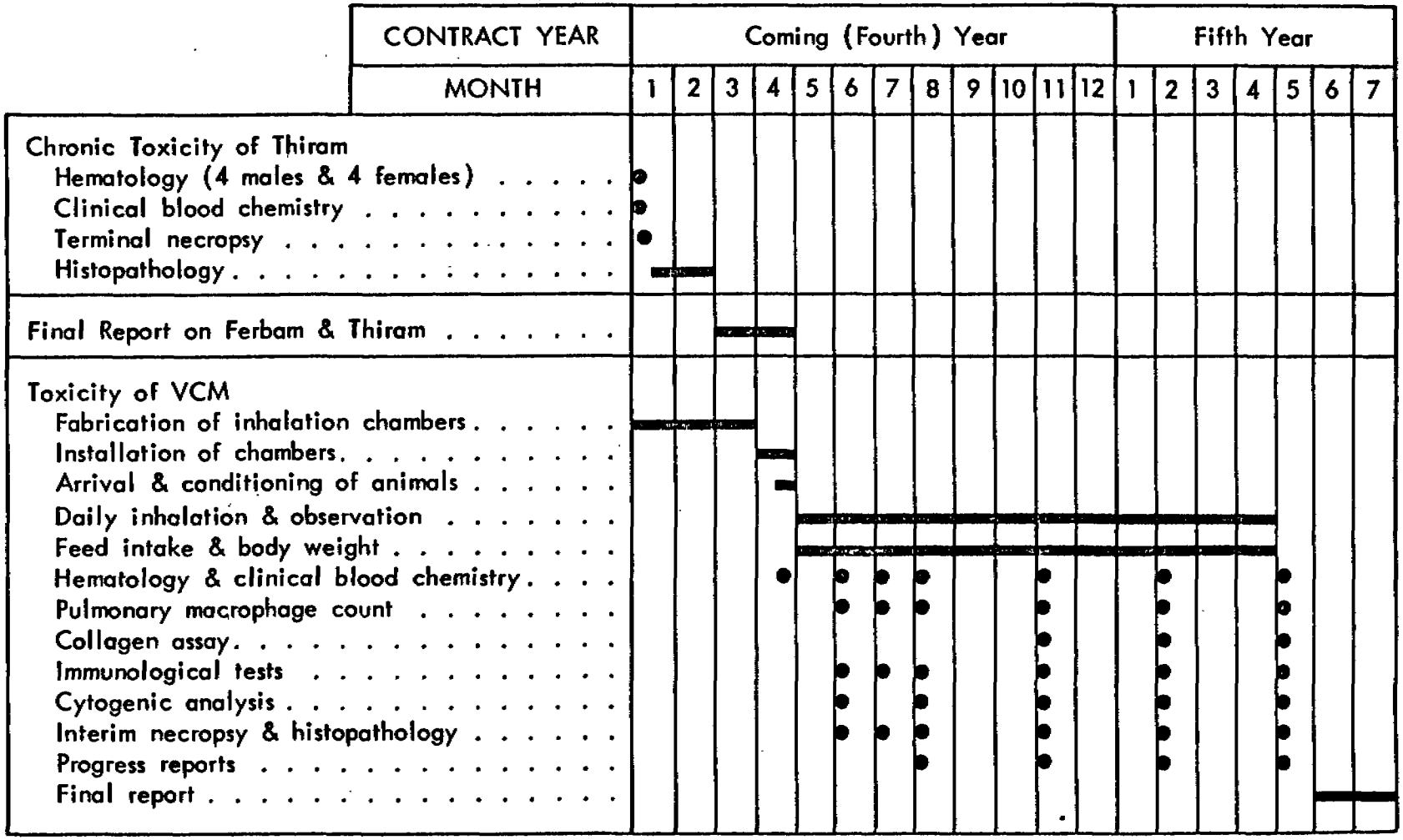
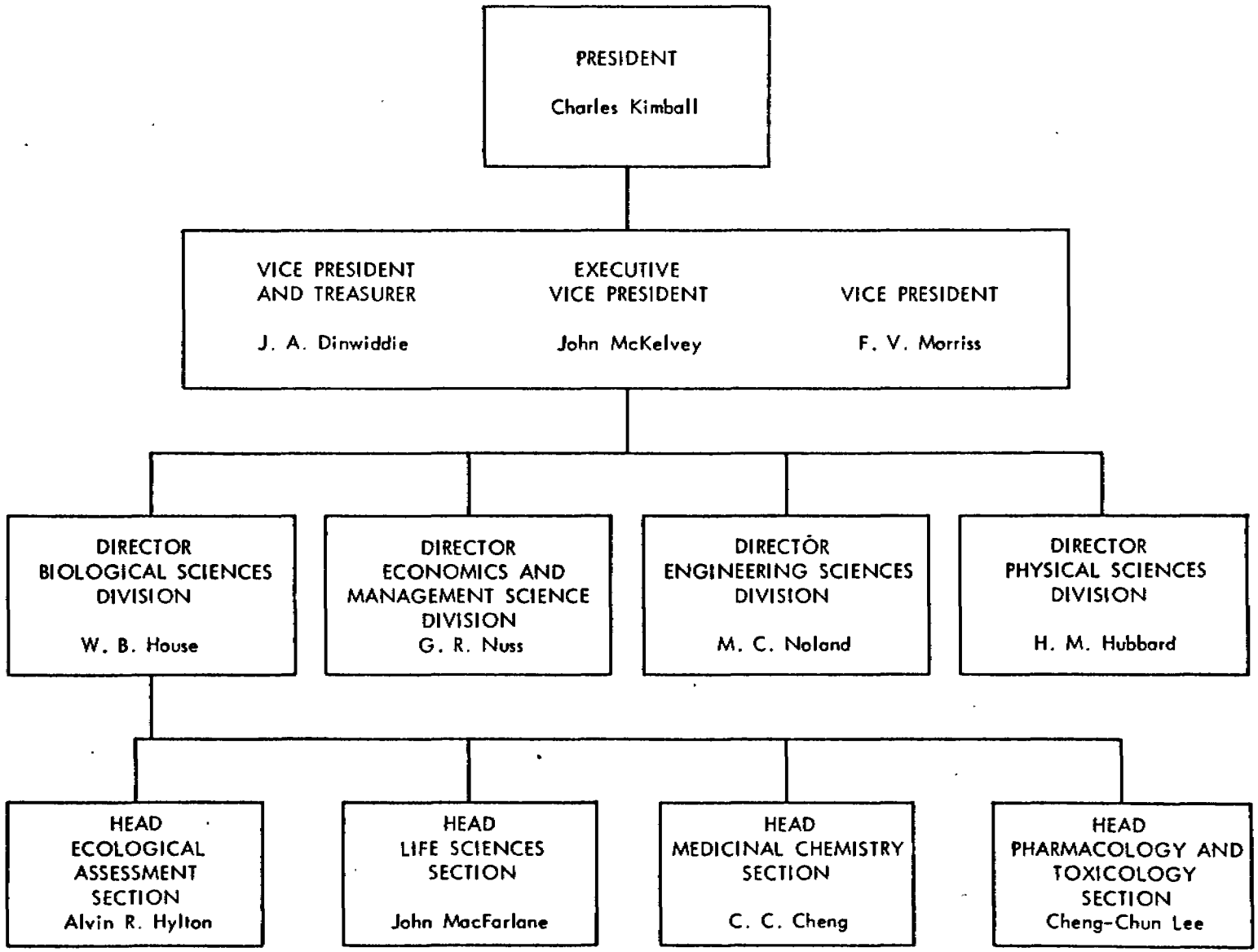


Figure 2 - Time Schedule for the Proposed Studies



MRI ORGANIZATION

Figure 3

R&S 131822

William B. House, Director, Biological Sciences Division: Dr. House has directed research projects involving chronic inhalation toxicity experimentation on primates and rodents; biochemical studies of the aging process, and the development of survival rations for extenuating circumstances. As Director, he directs the professional activities of a 60-man Biological Sciences staff. His duties include coordination and planning of research projects in the fields of pharmacology, toxicology, virology, biochemistry, physiology, microbiology, food technology and nutrition, and medicinal chemistry. The proposed program will be administered in the Biological Sciences Division. Dr. House will appoint a new Program Director with the approval of the Project Officer should the Program Director for the proposed studies be unavailable.

B. Technical Personnel

The staff of the Biological Sciences Division represents a wide range of skills and specialized experience to conduct research in biology, including pharmacology and toxicology. The pharmacology and toxicology group has the scientists, the equipped laboratories, the animal facilities and the experience to conduct studies in the following areas: drug absorption, distribution, metabolism and excretion; mechanisms of drug action; activities of and factors affecting microsomal drug-metabolizing enzymes; drug-drug interactions; factors affecting drug response; acute, subacute, chronic and teratogenic toxicities; mechanisms of toxicity; development of procedures for treating toxic effects of drugs; evaluation of chemicals for therapeutic use; and the pharmacology of new drugs.

R&S 131823

Scientists holding advanced degrees and experienced personnel from other sections of the Biological Sciences Division, as well as from other divisions routinely work on projects requiring the skills of more than one discipline.

1. Pharmacology and Toxicology

Only the staff assigned to the proposed project and those who will be available for assistance are listed below. Complete resumes of Drs. Lee, Dilley, Hodgson, Castles, Crawford and Rao are given in Appendix I.

Cheng-Chun Lee, Head, Pharmacology and Toxicology Section; B.S. in Veterinary Medicine, 1948; M.S. in Veterinary Medicine, 1948; National Central University, Chungking, China; M.S. in Pharmacology, 1950; and Ph.D. in Physiology, 1952, Michigan State University. Dr. Lee has more than 25 years experience in pharmaceutical research. He has conducted and/or directed research in toxicology and teratology, in pharmacodynamics, in absorption, excretion, distribution and metabolism of various drugs; in cholesterol metabolism and cholesterol-lowering compounds; in chemotherapy and

antimicrobial agents; in tracer technique; and in microsomal drug-metabolizing enzymes. Dr. Lee has published more than 70 scientific papers in various national and international scientific journals, and he is a member of several national scientific societies, including the American Society for Pharmacology and Experimental Therapeutics, the American Physiological Society, the Society of Toxicology, the New York Academy of Sciences, and others. Dr. Lee is on the research and teaching staff at the Department of Pharmacology, University of Kansas Medical School, Kansas City, Kansas. He will continue to be the Program Director and Principal Investigator of the proposed program.

James V. Dilley, Senior Toxicologist; B.S. in General Science, 1959, University of Portland; Ph.D. in Pharmacology-Toxicology, University of Chicago. Dr. Dilley has conducted research in acute, subacute and chronic toxicity of organophosphate and carbamate insecticides and herbicides; in deposition, retention and clearance of inhales particulates; in cigarette smoking; in inhalation toxicity of radioactive materials; in toxic hazards during space-flight and potential toxic effects of spacecraft materials and components. Dr. Dilley has published over 20 scientific papers in the field of toxicology and inhalation. He is a member of the Illinois Society for Medical Research, the American Public Health Association, the New York Academy of Science, the American Association for the Advancement of Science, and Sigma Xi. Dr. Dilley will assist the Principal Investigator and will be in charge of the inhalation chambers and of the various functional measurements.

John R. Hodgson, Associate Biochemist; B.S. in Chemistry, 1967; Ph.D. in Biophysics, 1970, University of Rhode Island. Dr. Hodgson joined the MRI staff in pharmacology and toxicology in 1971. His predoctoral training was primarily involved with studies of molecular interactions within subcellular particulates. In addition, Dr. Hodgson has had experience in electron microscopy under a post-doctorate program at Brown University. He has published in recognized scientific journals and is a member of the American Association for the Advancement of Science. He will perform the cytogenic study.

Thomas R. Castles, Principal Pharmacologist; B.A. in Chemistry-Zoology, 1959, Grinnell College; M.S. and Ph.D in Pharmacology, 1962 and 1965, University of Iowa. Dr. Castles has conducted research in renal function (clearance and transport systems); renal biochemistry (ATPase, carbonic anhydrase and nucleic acid synthesis); cardiovascular function (blood pressure screen, isolated heart, heart-lung and hind-limb perfusion); neuromuscular function; and the biochemistry of analgetic tolerance to narcotics. He has experience in performing acute, subacute and chronic toxicity tests of new antimalarial agents and has several publications in these areas. Dr. Castles is a member of the Society of Toxicology and the American Society for Pharmacology and Experimental Therapeutics, and is on the research and teaching staff at the Department of Pharmacology, University of Kansas Medical

R&S 131824

School, Kansas City, Kansas. He will assist the proposed studies when needed; and he has considerable experience in computer programs involving biological projects.

Chester R. Crawford, Senior Toxicologist; B.S. in Chemistry, 1949, University of Louisville; M.S. in Physiology, 1959, University of Iowa; Ph.D. in Toxicology, 1970, University of Kansas. Dr. Crawford has conducted research in acute, subacute, and chronic toxicity of organophosphate and carbamate insecticides, herbicides, other agricultural products, and antimalarials. He has been specially interested in studies on antidotes for organophosphate poisoning. He also has experience in histological techniques, experimental animal surgery, and analysis of steroids. Dr. Crawford has published several scientific papers and he is a member of the Society of Toxicology, Sigma Xi, and the Scientific Research Society of North America. Dr. Crawford will be available for assisting the proposed studies.

Robert D. Short, Jr., Associate Teratologist; B.S. in Biology, St. Lawrence University, 1964; Pharm.D. in Hospital Pharmacy, College of Pharmacy, University of Michigan, 1968; M.S. and Ph.D. in Pharmacology, Michigan State University, 1971 and 1973. Dr. Short had studied the toxicity of cyclophosphamide in perinatal mice and during fetal development. His research interests include the mechanism(s) by which the teratogenic effects are produced, the biochemistry of development under the influence of chemicals and environmented toxicants, drug metabolizing enzymes, drug absorption, disposition and metabolism. He has published a number of scientific papers and is a member of the Teratology Society and Society for Developmental Biology. Dr. Short will be available for assisting the proposed studies.

A. Kishman Rao, Associate Environmental Engineer; B.S.M.E., 1969, Osmania University, Andhra Pradesh, India; M.S. and Ph.D. in Mechanical Engineering, 1970 and 1974, University of Minnesota. Dr. Rao has been involved in the sampling and measurement of particulate and gaseous pollutants, and the analysis of air pollution data. He has been involved in the building of a mobil air pollution laboratory and the calibration of optical particle counters. He has studied the aerosols produced by the photochemical oxidation of SO_2 in air. He has been involved in the analysis of data from the Denver Atmospheric Aerosol Study, a Los Angeles Smog project, a study on cloud and ice nuclei, and a study of particle phenomena in relation to spray drying. Dr. Rao will assist in the calibration of the exposure chambers and the establishment and sampling of VCM atmospheres.

Thomas W. Reddig, Medical Technologist; B.S. in Biology and Chemistry, 1966, Kansas State Teachers College; M.T., 1971, Providence Hospital School of Medical Technology, Kansas City, Kansas. Mr. Reddig is a certified medical technologist by the ASCP. He was a medical technician in the U.S. Army for 3 years and medical technologist in the Providence-St. Margaret Health

Center, Kansas City, Kansas, for 2 years. At MRI, Mr. Reddig has worked on various projects involving hematology, clinical blood chemistry, and urinalysis. He will perform various laboratory tests and maintain up-to-date newer and better procedures for the various tests.

Judith Don Girvin, Medical Technologist; B.S. in Biology and Chemistry, 1970, Kansas State Teachers College; M.T., 1971, Providence Hospital School of Medical Technology, Kansas City, Kansas. Miss Girvin is a certified medical technologist by the ASCP. She was a medical technologist in the clinical laboratory of the Providence-St. Margaret Health Center, Kansas City, Kansas. At MRI, Miss Girvin has performed hematology, clinical blood chemistry and urinalysis for various projects.

John J. Kowalski, Junior Biologist; B.S. in Biology-Environmental Studies, 1972, University of Kansas, Lawrence, Kansas. Mr. Kowalski has several years experience in laboratory animals during his college education. At MRI, he has performed various animal experiments and gained experience in various phases of toxicity experimentation including the use of rodents, beagles and rhesus monkeys. He will assist animal experimentation.

Bruce S. Anderson, Junior Biologist; B.S. in Biology-Chemistry, 1973, University of Kansas. Mr. Anderson has several years experience in laboratory animals. At MRI, he has performed various animal experiments and gained experience in various phases of toxicity experimentation. He will assist animal experimentation and various functional measurements for the proposed studies.

Ernesto A. Castillo, Junior Biologist; B.S. in Animal Husbandry, 1962, Araneta University, Philippines; study in Veterinary Medicine, 1962-1967, University of Philippines. Mr. Castillo had performed routine veterinary care to livestocks and pets, and assisted surgery, radiology and clinical pathology under the supervision of the veterinarian. At MRI, he has assisted necropsy of various laboratory animals and has had extensive experience in histological techniques. He will assist necropsy and prepare tissue specimens for histopathology for the proposed studies.

2. Veterinary Medicine and Pathology

Jaime L. Sanyer, Associate Pathologist; D.V.M., University of Guayaquil, Ecuador, 1962; M.S. in Toxicology and Veterinary Pathology, 1972, Kansas State University. Dr. Sanyer was a clinical veterinarian in charge of the monkey colony at the Institute of Comparative Medicine, Columbia University, New York. He has experience in using primates for research, to perform necropsies on laboratory animals, and in examining tissues of histopathology. He has several scientific publications and is a member of the American Veterinary Medical Association and a fellow of the American College

of Veterinary Toxicologists. Dr. Sanyer will perform necropsy and gross examination, supervise tissue preparation, and perform microscopic examination.

3. Consultants

Loren D. Kintner, D.V.M.; Consultant in Veterinary Pathology for MRI; Professor in Veterinary Pathology, Department of Veterinary Pathology and Veterinary Diagnostic Laboratory, School of Veterinary Medicine, University of Missouri, Columbia, Missouri. Dr. Kintner has extensive experience in veterinary pathology and in pathological diagnoses. He is a board-certified veterinary pathologist and has numerous scientific publications. He has been the principal veterinary pathologist for several projects at MRI. During the past several years, Dr. Kintner has come to the Institute and performed necropsies on beagles and other animals at termination of the experiments. He and his associates have performed microscopic examination on several hundred beagles and primates and several thousand rodents annually for MRI. Dr. Kintner will consult on gross and microscopic examination of tissues.

Harlen E. Jensen, D.V.M., Ph.D., Consultant in Veterinary Ophthalmology for MRI; Professor and Chief in Ophthalmology, Department of Veterinary Medicine and Surgery, School of Veterinary Medicine, University of Missouri, Columbia, Missouri. Dr. Jensen is board certified and has extensive experience in veterinary ophthalmology. He is the author of the Stereoscopic Atlas of Clinical Ophthalmology of Domestic Animals. Dr. Jensen has performed ophthalmological examination of beagles, monkeys, and rodents for several projects at MRI. He will consult on ophthalmological examination of animals for the proposed studies.

4. Statisticians and Analysts

Michael C. Sharp, Associate Statistician; B.S. in Physics, 1965; M.S. in Applied Statistics, 1968, University of Missouri at Rolla. Mr. Sharp specializes in experimental design, regression and correlation studies, survey design, data reduction, specialized sampling techniques, and quality control. He has been principal investigator on several projects involving statistical analysis, such as research in medical diagnosis efficacy and in manufacturing quality control. Most of his efforts, however, are spent as a project team member responsible for the experimental design and statistical analysis in a wide variety of research fields. The diversity of his work is exemplified by his contributions to projects in traffic engineering, environmental and pollution studies, biological and medical investigations, and economic and management science programs.

Christine Guenther, Analyst; B.S. in Mathematics, University of Missouri at Kansas City, 1968; additional courses at the same university.

R&S 131827

Joining the staff of MRI in 1968, Miss Guenther has experience in mathematical modelling and mathematical and statistical analysis of biological, chemical, and engineering data. She has designed and applied computer programming techniques for the reduction and analysis of these data. She has also programmed output for a highway vehicle simulation and has been involved with the application of NASA-developed science and technology to other scientific disciplines. Miss Guenther has a working knowledge of FORTRAN, BASIC, COBOL, and assembler programming languages. She is a member of the Society of Industrial and Applied Mathematics.

5. Animal Handlers

We have several experienced animal handlers who have worked with various animals for many years. We feel that the experienced handlers are most necessary for any long-term studies especially employing beagles and rodents.

Edward Williams, Animal Handler Supervisor. Mr. Williams has more than 10 years experience with various laboratory animals. He thoroughly understands the behavior and habits of these animals. Since joining MRI, he has been in charge and directs our entire animal quarters and animal care operation. Through our safety evaluation studies on investigational antimalarial agents during the past several years, he has had additional experience in handling and administering compounds for long-term studies.

VII. Previous Experience and Current Research Projects

Tolerance Criteria for Continuous Inhalation Exposure to Toxic Materials: During the first part of the program, clinical laboratory and histopathological changes in 50 monkeys, 250 rats and 500 mice were studied during continuous 90-day exposure to controlled atmospheres of phenol, CCl_4 and a mixture of indole, skatole, H_2S and methyl mercaptan. During the second part of the program, clinical laboratory and histopathological changes in 50 monkeys, 250 rats and 500 mice were studied during continuous 90-day exposure to controlled atmospheres of H_2S, methyl mercaptan, indole and a mixture of H_2S, methyl mercaptan, indole and skatole. During the third part of the program the toxic effects of maximum allowable concentrations of hydrazine, unsymmetrical dimethylhydrazine, decaborane and nitrogen dioxide during continuous 90-day exposure were studied in 40 monkeys, 200 rats and 400 mice.

This work was performed under:

Contract No. AF 33(616)-7955, "Tolerance Criteria for Continuous Inhalation Exposure to Toxic Materials. I. Effects on Animals of 90-Day

R&S 131828

Exposure to Phenol, CCl_4 and a Mixture of Indole, Skatole, H_2S, Methyl Mercaptan. II. Effects on Animals of 90-Day Exposure to H_2S, Methyl Mercaptan, Indole and a Mixture of H_2S, Methyl Mercaptan, Indole and Skatole." WADD.

Contract No. AF 33(657)-8505, "Tolerance Criteria for Continuous Inhalation Exposure to Toxic Materials. III. Effect on Animals of 90-Day Exposure to Hydrazine, Unsymmetrical Dimethylhydrazine, Decarborane and Nitrogen Dioxide." WADD.

Contact may be made with Dr. Kenneth Back, Chief, Toxicology Branch, Aerospace Medical Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio, Telephone 513-255-5588.

Metabolism and Pharmacology of TAMA and AMOX: In this program, inhalation, oral and intraperitoneal toxicities of the two chemical compounds were studied. More than 15 tissues from each animal were examined for the chemical content. There were more than 2,500 fluorine determinations made in the study. In addition, a large number of hematological and urinological evaluations were made.

The work was performed under Contract No. AF 33(615)-2472, "Metabolism and Pharmacology of TAMA and AMOX," Systems Engineering Group, Wright-Patterson AFB. Contact may also be made with Dr. Kenneth Back of Aerospace Medical Laboratories, Wright-Patterson AFB, Ohio, Telephone 513-255-5588.

Safety Evaluation of Candidate Antimalarial Compounds: The major emphasis of this project has been the toxicological evaluation of candidate antimalarial compounds in rodents, beagles, and monkeys. The acute, subacute, and/or chronic toxicities of candidate compounds are studied. The effects of these compounds on gross observation, hematology, functional tests, and urinalysis are investigated. Information is coded and recorded on IBM cards which in turn are stored for statistical analysis of significant difference among groups and for later retrieval purpose. At termination, necropsy is performed for gross examination; various tissues are removed, preserved, and prepared for microscopic examination. In addition, specific tests are designed to elucidate the mechanism(s) of toxicity, reversibility, combination toxicity and interaction, reproductive, and teratological effects of candidate compounds. During the past 10 years, some 95 separate studies have been completed. These studies cover a wide range of investigations on the old and new candidate compounds.

The work is performed under Contract No. DA-49-193-MD-2759, "Toxicological and Pharmacological Evaluation of Antimalarial Compounds," U.S. Army Medical R&D Command, Office of the Surgeon General. Contact may be made with Dr. Melvin H. Heiffer or Dr. Robert S. Rozman, Chief and Deputy

R&S 131829

Chief of the Department of Pharmacology, Division of Medicinal Chemistry, Walter Reed Army Institute of Research, Walter Reed Medical Center, Washington D.C., Telephone 202-576-3387.

Preclinical Toxicologic Study of Anticancer Agents: This project is concerned with the toxicological evaluation of candidate anticancer agents prior to clinical evaluation. Specifically, the research is to determine the acute and subacute toxicities of the candidate compounds in beagle dogs and rhesus monkeys; to establish the maximum tolerated dose, toxic dose low, toxic dose high, and lethal dose; and to investigate test procedures for predicting their toxicities in man. During the first 2 years, we studied five anticancer agents including cyclophosphamide and cyclocytidine. Currently, we have been investigating two new candidate compounds.

This work is performed under Contract No. NIH-71-2263, "Preclinical Toxicologic Study of Anticancer Agents," National Cancer Institute, NIH. Contact may be made with Dr. Anthony M. Guarino, Chief, Laboratory of Toxicology, National Cancer Institute, NIH, Bethesda, Maryland, Telephone 301-594-6713.

Mammalian Toxicity of Munition Compounds: This project is concerned with an extensive evaluation of the long-range toxicological effects of munition compounds that find their way into waste water from the manufacture of army ammunition. These compounds include nitroglycerin and its mono- and di-nitro isomers, trinitrotoluene and its di-nitro isomers, nitrocellulose, and white phosphorus. Acute toxicity, primary irritation and skin sensitization are studied in mice, rats, rabbits, and guinea pigs. Subacute and chronic toxicity of the test compounds and reversibility of both the biochemical and subcellular lesions are studied in rodents and dogs. Assessment of carcinogenicity is performed in all test species. Three generation reproduction studies, including two mutagenic-teratogenic studies, are performed in rats. Independent mutagenic studies include the cytological test and specific locus mutations test. Absorption and distribution of isotope-labeled compounds are studied in rats. The metabolites are isolated and identified.

This project is performed under Contract No. DAMD-17-74-C-4073, "Munition Compounds Mammalian Toxicity Study," Environmental Protection Research Division, U.S. Army Medical R&D Command, Washington, D.C. Contact may be made with Cpt. Glennon, EPRD, USAMRDC, Washington, D.C., Telephone 202-693-8061.

Bioavailability of Lead in Shellfish: This project is concerned with the toxicity in rats fed lead-ladened oysters. Oyster meats from oysters grown in lead acetate solution, normal (low lead) oyster meats, and normal oyster meats mixed with comparable quantities of lead acetate, will be compared using bioassay techniques. Particular attention will be given

to standardizing all dietary constituents (especially zinc, copper and iron). Assays will include body weights, feed consumption, hematology, and lead body-burden.

This project is performed under Contract No. FDA-73-246, "Bioavailability of Lead in Shellfish," Shellfish Sanitation Branch, FDA, Washington, D.C. Contact may be made with Dr. T. J. Sobotha, Psychotoxicity Unit, Food and Drug Administration, Department of Health, Education and Welfare, Washington, D.C. Telephone 202-245-1304.

R&S 131831

Brain Chromatin - Its Role in Analgesic Tolerance: This project is concerned with the relationship of analgesic tolerance and a morphine-induced change in rat brain chromatin. These studies include identification of changes in chromosomal proteins, changes in RNS using RNA-DNA hybridization and examination of morphine bending to chromatin from tolerant rats.

This project is performed under PHS Grant No. DA-00934, National Institute on Drug Abuse, Rockville, Maryland.

VIII. Facilities and Equipment

A. General

The Midwest Research Institute's main building, which contains 150,000 sq ft of floor space with approximately 100 completely equipped laboratories, and more than $1,500,000 worth of scientific and computing equipment, adjoins both the campus of the University of Missouri at Kansas City and the Linda Hall Library of Science and Technology. A new addition to the main building was completed in May of 1971, and adds 45,000 sq ft of floor space. The biological sciences, chemistry, physics, and engineering laboratories are completely equipped with the most modern research tools.

The facilities of Linda Hall Library of Science and Technology will be available for any literature research required. Linda Hall Library, one of the largest technical libraries in the world, has a collection of approximately 350,000 volumes of scientific works, and receives more than 11,000 current periodicals in science and technology in 36 languages. It is one of the 12 Federal Regional Technical Report Centers for unclassified government research reports and a repository for the U.S. Patent Office.

The Clendening Medical Library of the School of Medicine of the University of Kansas in Kansas City, Kansas, is also readily accessible. This library has approximately 68,000 volumes on medicine and receives 1,200 serial publications.

B. Pharmacology and Toxicology

1. Facilities and Equipment

The facilities and cognate equipment in Pharmacology and Toxicology, in which the proposed studies will be performed and administered, comprise the following laboratories:

Hematology and clinical chemistry
Histology and pathology
Teratology
Mutagenesis and tissue culture
Drug metabolism
Molecular biology
Inhalation
Animal behavior

2. Animal Quarters

The Institute handles animal experimentation in two areas, the Institute's main complex and the Deramus Field Station. All animal quarters are air-conditioned and humidity-controlled.

R&S 131832

a. Main complex

(1) Small animals: On the third floor of the main building, there is an area of approximately 2,000 sq ft with eight animal rooms. These facilities are used for acute and subacute studies and can accommodate 4,000 mice, 600 rats, 200 guinea pigs, and 100 rabbits. With these animal quarters, there are auxiliary facilities for cleaning and storage.

On the first floor of the main building, there is an area of approximately 1,000 sq ft with five animal rooms. These facilities are used for chronic studies and are equipped with modern air handling and filter systems. The authorized personnel enter these rooms through a shower and locker room where they can put on overalls, caps, mouth masks, and shoe covers. Access to the "dirty" corridor is through one-way doors. The rodents will be housed in filter-top plastic cages to reduce any possible cross infection among individual animals. Hardwood chips used as bedding material for these rats will be sterilized and changed weekly. There are auxiliary facilities for necropsy, cleaning and storage for both the small and large animals.

(2) Large animals: On the first floor of the main building there is an area of 1,000 sq ft with five animal rooms. These rooms will house a total of 84 double-tier dog cages (28 in. x 34 in. x 28 in.). All cages are made of stainless steel with combination metabolism and

draining pans. The cages can be flushed with a "water gun" and waste material collected in draining troughs located along the backs of the cages and carried into the sewer. By converting the dog cages, these rooms can be used to house monkeys.

On the first floor of the Spencer Building, there is an area of 7,000 sq ft with five dog rooms, four monkey rooms, and treatment, necropsy, cleaning and storage rooms. Authorized personnel enter this area through a shower and locker room into a middle "clean" corridor. They leave these rooms to the "dirty" corridor through one-way doors. The relative humidity of the monkey rooms is kept at 70 ± 5% and that of the dog rooms is kept at 50 ± 5%.

b. Deramus Field Station: The Deramus Field Station is a 75-acre facility located 15 miles from the main complex. This facility has several buildings for biological research, including the following:

(1) Toxicology Research Building: This building provides 18 dog pens complete with outside runs, and two animal rooms for rodents. The pens and runs can accommodate a maximum of 288 dogs. Auxiliary space includes necropsy, a laboratory, cleaning and storage rooms, and an office. These facilitiea are used for subacute and chronic toxicity studies.

(2) Speas Memorial Laboratory: This building has an area of 3,000 sq ft with five rodent rooms, an office, and cleaning and storage rooms. One-way traffic from the "clean" corridor through the animal rooms to the "dirty" corridor is arranged in this building for authorized personnel.

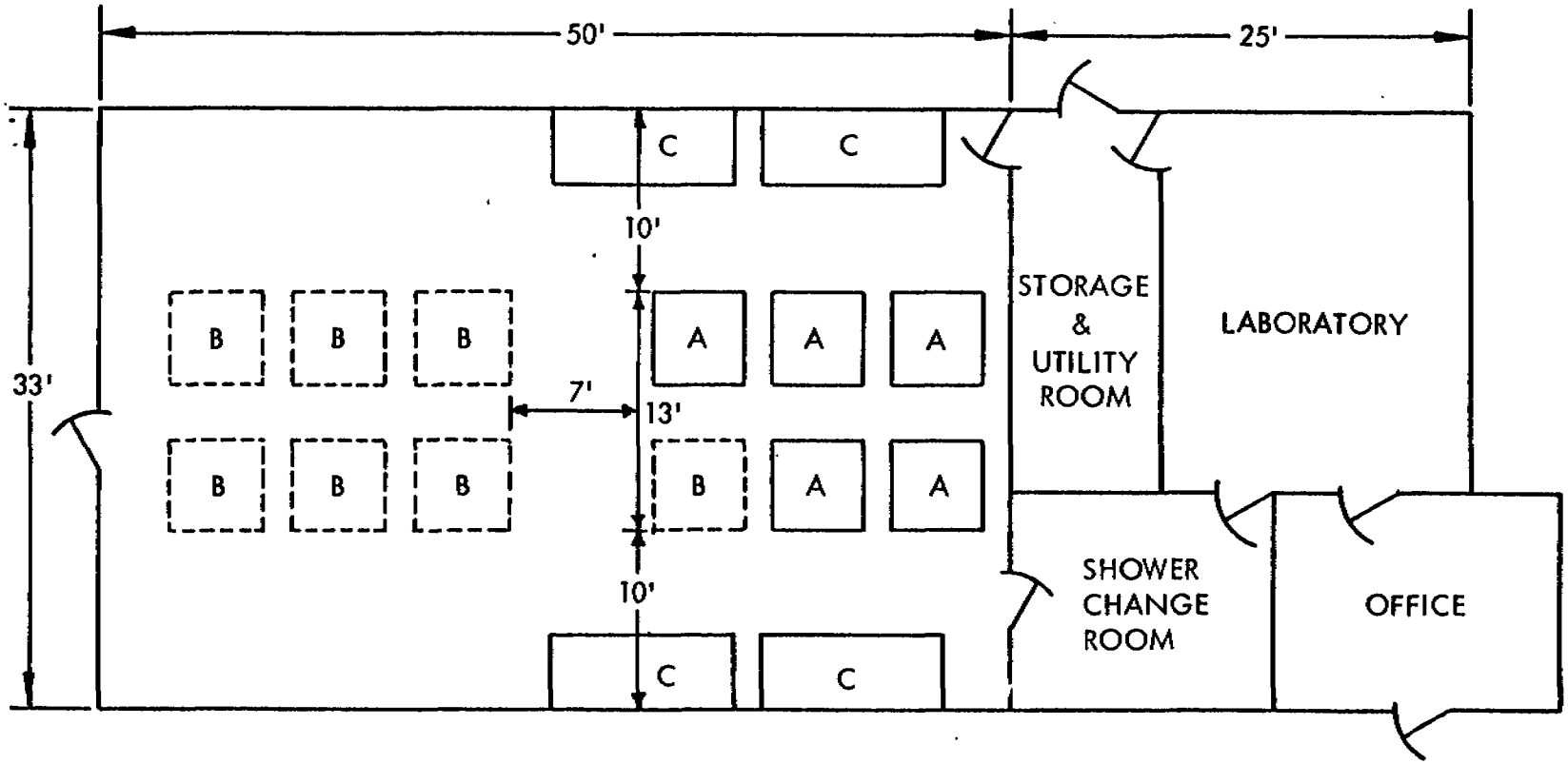
(3) Kelce Memorial Laboratory: This building has a total area of 2,400 sq ft. This building and the Speas Memorial Laboratory have accommodated 30,000 mice involved in a study of carcinogenesis.

C. Facilities and Equipment for the Proposed Studies

The inhalation facilities for the proposed studies will be performed in the Kelce Memorial Laboratory at the Deramus Field Station. A drawing of the floor plan is shown in Figure 4. There will be five inhalation chambers (54 in. x 54 in. x 54 in.) located in the middle of the inhalation room. Four chambers will be needed for the proposed studies and one chamber will be available for any emergency. Four animal holding chambers on casters will be located on both sides of the room. As seen on the floor plan, additional chambers can be installed when they are needed.

There is ample space in this building for various operations of the animals including blood collection, functional measurements, and necropsy.

R&S 131833



A. 54" x 54" inhalation chambers to be used for VCM exposures
B. Locations for additional chambers in the future
C. Ventilated portable holding chambers for animals when they are not being exposed

Figure 4 - Floor Plan of Kelce Memorial Laboratory at the Deramus Field Station

R&S 131834

R&S 131835

Hematology, clinical blood chemistry, biochemical assay, histological preparation, and other special studies will be performed in this building or in the laboratories at the main complex of the Institute.

IX. Estimated Cost

It is estimated that the work as described for the coming contract year will require $163,370, including a fixed fee of $9,248. Details of the budget are shown on the enclosed optional Form 60. Because MRI expects approximately $55,570 of current contract funds to remain unexpended at the end of February, 1975, we estimate the need for additional funding in the amount of $107,800.