SECTION I

Form Approved
Budget Bureau No. 68-R0249

DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

GRANT APPLICATION

LEAVE BLANK

TYPE	PROGRAM	NUMBER
REVIEW GROUP		FORMERLY
COUNCIL (Month, Year)		DATE RECEIVED

TO BE COMPLETED BY PRINCIPAL INVESTIGATOR (Items 1 through 7 and 15A)

1. TITLE OF PROPOSAL (Do not exceed 53 typewriter spaces)
Experimental Asbestos Carcinogenesis

2. PRINCIPAL INVESTIGATOR

2A. NAME (Last, First, Initial)
Reeves, Andrew L.

2B. TITLE OF POSITION
Professor

2C. MAILING ADDRESS (Street, City, State, Zip Code)
625 Mullett Street
Detroit, Michigan 48226

2D. DEGREE
Ph. D.

2E. SOCIAL SECURITY NO.
374-36-6539

2F. TELEPHONE DATA — Area Code: 313 — TELEPHONE NUMBER AND EXTENSION: 577-1224

2G. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT (See Instructions)
Dept. of Occupational & Environ. Health

2H. MAJOR SUBDIVISION (See Instructions)
School of Medicine

3. DATES OF ENTIRE PROPOSED PROJECT PERIOD (This application)
FROM: 8-1-1973 — THROUGH: 7-31-1977

4. TOTAL DIRECT COSTS REQUESTED FOR PERIOD IN ITEM 3
$315,~~250~~ 550

5. DIRECT COSTS REQUESTED FOR FIRST 12-MONTH PERIOD
$76,915

6. PERFORMANCE SITE(S) (See Instructions)
Wayne State University School of Medicine
Department of Occupational and
Environmental Health
625 Mullett Street
Detroit, Michigan 48226
13th Congressional District

7. Research Involving Human Subjects (See Instructions)
A. [illegible]
C. ☐ YES — Pending Review — Date

8. Inventions (Renewal Applicants Only - See Instructions)
A. ☐ NO B. ☐ YES — Not previously reported
C. ☐ YES — Previously reported

TO BE COMPLETED BY RESPONSIBLE ADMINISTRATIVE AUTHORITY (Items 8 through 13 and 15B)

9. APPLICANT ORGANIZATION(S) (See Instructions)
Wayne State University
5050 Cass Avenue
Detroit, Michigan 48202
IRS I.D. #69-0353-877

10. NAME, TITLE, AND TELEPHONE NUMBER OF OFFICIAL(S) SIGNING FOR APPLICANT ORGANIZATION(S)
Donald Kilpela
Acting Director
Grant and Contract Administration
Telephone Number (s) (313) 577-2291

11. TYPE OF ORGANIZATION (Check applicable item)
☐ FEDERAL ☒ STATE ☐ LOCAL ☐ OTHER (Specify)

12. NAME, TITLE, ADDRESS, AND TELEPHONE NUMBER OF OFFICIAL IN BUSINESS OFFICE WHO SHOULD ALSO BE NOTIFIED IF AN AWARD IS MADE
Pat Woodruff
Fiscal Officer
1094 Mackenzie Hall
Detroit, Michigan 48202
Telephone Number (313) 577-2283

13. IDENTIFY ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR INSTITUTIONAL GRANT PURPOSES (See Instructions)
01 School of Medicine

14. PHS ACCOUNT NUMBER (Enter if known)
639352

15. CERTIFICATION AND ACCEPTANCE. We, the undersigned, certify that the statements herein are true and complete to the best of our knowledge and accept, as to any grant awarded, the obligation to comply with Public Health Service terms and conditions in effect at the time of the award.

SIGNATURES (Signatures required on original copy only. Use ink. "Per" signatures not acceptable)		DATE
	A. SIGNATURE OF PERSON NAMED IN ITEM 2A	1-26-73
	B. SIGNATURE(S) OF PERSON(S) NAMED IN ITEM 10	1-29-73

PHS-398
Rev. 3-70

8000 1133

80.52

HFM - 002075

SCF-FORD-0979

SECTION 1

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

RESEARCH OBJECTIVES

LEAVE BLANK

PROJECT NUMBER

NAME AND ADDRESS OF APPLICANT ORGANIZATION

Wayne State University, 5050 Cass Avenue, Detroit, Michigan 48202

NAME, SOCIAL SECURITY NUMBER, OFFICIAL TITLE, AND DEPARTMENT OF ALL PROFESSIONAL PERSONNEL ENGAGED ON PROJECT, BEGINNING WITH PRINCIPAL INVESTIGATOR

Andrew L. Reeves, Ph. D., 374-36-6539, Professor, Dept. Occup. Environ. Health

Henry E. Puro, M. D., 364-26-8470, Associate Professor, Dept. Pathology

Neil D. Krivanek, Ph. D., 396-42-6572, Instructor, Dept. Occup. Environ. Health

Anil K. Roy-Chowdhury, Ph. D., 494-60-5461, Research Associate, Dept. Occup. Environ. Health

TITLE OF PROJECT

Experimental Asbestos Carcinogenesis

USE THIS SPACE TO ABSTRACT YOUR PROPOSED RESEARCH. OUTLINE OBJECTIVES AND METHODS. UNDERSCORE THE KEY WORDS (NOT TO EXCEED 10) IN YOUR ABSTRACT.

Animal experiments will be performed in order to identify the carcinogenic principle(s) in asbestos fibers. The mechanical irritation theory will be tested by comparing the effects of pleural asbestos deposits under conditions of normal respiratory movements vs. conditions of rest (pneumothorax). The trace metal theory will be tested by studying the effects of asbestos specimens from which carcinogenic metals were removed. The organic contaminant theory will be tested by studying the effects of solvent-washed specimens. Specimens will be tested both by intraperitoneal injection and inhalation. Improved chamber dissemination system will be built in which fiber destruction and trace metal contamination is minimized. The effects of heat treatment of asbestos on its biological activity will be explored, and an automotive brake emission generator will be built to test the effects of brake dust inhalation on experimental animals. The question of species specificity in asbestos [illegible] exposure.

LEAVE BLANK

SECTION II – PRIVILEGED COMMUNICATION

DETAILED BUDGET FOR FIRST 12-MONTH PERIOD

FROM: 8-1-73 THROUGH: 7-31-74

DESCRIPTION (Itemize)		TIME OR EFFORT DEVOTED TO PROJECT	AMOUNT REQUESTED (Omit cents)		
PERSONNEL					
NAME	TITLE OF POSITION	%	SALARY	FRINGE BENEFITS	TOTAL
Andrew L. Reeves	PRINCIPAL INVESTIGATOR	25	-0-	-0-	-0-
Henry E. Puro	Associate Professor	25	-0-	-0-	-0-
Neil D. Krivanek	Instructor	25	-0-	-0-	-0-
Anil K. Roy-Chowdhury	Research Associate	75	7,776	1,350	9,126
Duane VanAcker	Instrument Technician	75	10,238	2,112	12,350
Leonard Pickett	Histology Technician	75	8,808	1,950	10,758
Walter Dybicki	Veterinary Technician	100	11,410	2,218	13,628
(to be appointed)	Engineer	50	7,500	1,250	8,750
Genevieve Rainwater	Secretary	25	2,500	225	2,725
	TOTALS		48,232	9,105	57,337
CONSULTANT COSTS	R. L. Gealer and A. Anderson, Ford Motor Company Engineering Laboratories, to help build the dynamometric brake emission dissemination system				-0-
EQUIPMENT	Hardware to build dynamometer:				
	12 hp motor with installation			$ 750	
	2 solenoid valves and loading devices			1500	
	2 cycle timers and minor constituents			1000	
	Chamber and dissemination system hardware			750	
	Biosonic IV Cell Disruption Probe[1]			1500	
	TOTAL				5,500
SUPPLIES	Animals (4 mini-pigs, 250 guinea pigs, 500 rats)			2600	
	Animal feed and cage supplies[2]			2900	
	Chamber and dissemination system supplies[3]			600	
	Chemistry laboratory supplies			1200	
	Histopathology laboratory supplies			1200	
	TOTAL				8,500
TRAVEL	DOMESTIC To visit other research centers and conferences				600
	FOREIGN				-0-
PATIENT COSTS (See instructions)					0
ALTERATIONS AND RENOVATIONS					-0-
OTHER EXPENSES (Itemize)	Animal caretaker overtime (weekend service)			1878	
	Hourly pay for graduate students[4]			750	
	Service contracts and equipment repair[5]			1150	
	Publication costs[6]			600	
	Laundry, office supplies, telephone			600	
	TOTAL				4,978
TOTAL DIRECT COST (Enter on Page 1, Item 8)					76,915

INDIRECT COST (See instructions): 55% % S&W* % TDC* DATE OF DHEW AGREEMENT: 8-18-71 ☐ WAIVED ☐ UNDER NEGOTIATION WITH:

*IF THIS IS A SPECIAL RATE (e.g. off-site), SO INDICATE.

8000 1135

HFM - 002077

BUDGET ESTIMATES FOR ALL YEARS OF SUPPORT REQUESTED FROM PUBLIC HEALTH SERVICE
DIRECT COSTS ONLY (Omit Cents)

DESCRIPTION		1ST PERIOD (SAME AS DETAILED BUDGET)	ADDITIONAL YEARS SUPPORT REQUESTED (This application only)					
			2ND YEAR	3RD YEAR	4TH YEAR	5TH YEAR	6TH YEAR	7TH YEAR
PERSONNEL COSTS		57,337	61,924[7]	66,878[7]	72,228[7]			
CONSULTANT COSTS (Include fees, travel, etc.)		0	0	0	0			
EQUIPMENT		5,500	3,500[8]	0	0			
SUPPLIES		8,500	6,200[9]	5,200[9]	4,200[9]			
TRAVEL	DOMESTIC	600	600	600	600			
	FOREIGN	0	0	800[10]	0			
PATIENT COSTS		0	0	0	0			
ALTERATIONS AND RENOVATIONS		0	0	0	0			
OTHER EXPENSES		4,978	5,129[7]	5,289[7]	5,487[7]			
TOTAL DIRECT COSTS		76,915	77,353	78,767	82,515			

TOTAL FOR ENTIRE PROPOSED PROJECT PERIOD (Enter on Page 1, Item 4) ⟶ $ 315,250

REMARKS: *Justify all costs for the first year for which the need may not be obvious. For future years, justify equipment costs, as well as any significant increases in any other category. If a recurring annual increase in personnel costs is requested, give percentage. (Use continuation page if needed.)* 1. Ultrasonic instrument for tissue disruption--to prepare pulmonary tissue homogenates with preservation of ferruginous bodies. From VWR Scientific Co.

2. Rat chow $320, guinea pig chow $900, pig chow $180, Sani-Kleen $200, cage board $900, cedar shavings $300, miscellaneous $100.

3. Brake shoes will be donated to us from the Bendix Corporation.

4. Participating graduate students will receive $2.50/hr.

5. Service contract on x-ray diffraction apparatus ($250), 50% on atomic absorption spectrophotometer ($150), densitometric integrator ($125), 25% on electron microscope ($375); other repair contingencies, $250.

6. Page charges, reprints, medical art work, microphotography.

7. Computed at [redacted] annual across-the-board raises and/or average merit raises, including overtime payments.

8. Dissemination equipment and chamber alteration cost for modified dust inhalation experiments.

9. Decreasing amounts for animal purchases and supplies; $3,000 per annum throughout for laboratory and shop supplies.

10. Participation at the 1975 Inhaled Particles Conference, Edinburgh, and 18th Internat. Congress Occup. Health, Brighton.

8000 P136

SECTION II – PRIVILEGED COMMUNICATION

BIOGRAPHICAL SKETCH

(Give the following information for all professional personnel listed on page 2, beginning with the Principal Investigator. Use continuation pages and follow the same general format for each person.)

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Andrew L. Reeves	Professor	10-13-1924

PLACE OF BIRTH (City, State, Country)	PRESENT NATIONALITY (If non-U.S. citizen, indicate kind of visa and expiration date)	SEX
Budapest, Hungary	USA	☒ Male ☐ Female

EDUCATION *(Begin with baccalaureate training and include postdoctoral)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	SCIENTIFIC FIELD
University of Budapest, Hungary	B. S. equiv.	1949	Chemistry
University of Munich, Germany	Dipl. Chem.	1953	Organic Chemistry
Wayne State University, Detroit, Mich.	Ph. D.	1959	Biochemistry

HONORS

Member, Permanent Commission & International Association of Occupational Health
Fellow, American Institute of Chemists
NATO Senior Science Fellow, University of Milano, Italy

MAJOR RESEARCH INTEREST	ROLE IN PROPOSED PROJECT
Biochemistry, Toxicology	Principal Investigator

RESEARCH SUPPORT *(See instructions)*

U. S. Public Health Service Grant 2-RO1-ES 00353, "The Pathogenesis of Pulmonary Berylliosis", 6-1-72 to 5-31-76, total support $188,276, current year $44,100, 25% of time.

U. S. Public Health Service Grant 2-RO1-EC 00240, "Experimental Asbestos Carcinogenesis", 8-1-71 to 7-31-73, total support $121,392, current year $58,050, 25% of time.

RESEARCH AND/OR PROFESSIONAL EXPERIENCE *(Starting with present position, list training and experience relevant to area of project. List all or most representative publications. Do not exceed 3 pages for each individual.)*

1972-present Professor, Dept. of Occup. Environ. Health, Wayne State U.
1968-1972 Associate Professor, Dept. of Occup. Environ. Health, Wayne State U.
1963-1968 Assistant Professor, Dept. of Occup. Environ. Health, Wayne State U.
1956-1963 Research Associate, Dept. of Occup. Environ. Health, Wayne State U.
1954-1955 Research Assistant, Detroit Institute for Cancer Research.

Publications:

[redacted]

Desulfhydrase by Rat Liver Microsomes. Proc. Soc. Exp. Biol. Med. 88 (4), 594-596 (1955).

3. Reeves, A. L., Smith, R. G., and Vorwald, A. J.: The State of Beryllium in Blood Plasma. Fed. Proc. 17 (1), 1785 (1958).

4. Vorwald, A. J., and Reeves, A. L.: Pathologic Changes Induced by Beryllium Compounds--Experimental Studies. Arch. Indust. Health 19 (2), 190-199 (1959).

(Cont'd)

8000 1137 PQ

U. S. GOVERNMENT PRINTING OFFICE : 1971 O - 451-786

HFM - 002079

Publications Cont'd (Dr. A. L. Reeves)

5. Vorwald, A. J., and Reeves, A. L.: Inhaled Atmospheric Pollutants in the Genesis of Lung Cancer--An Experimental Study of Biochemical Changes Induced by Beryllium. Acta Union Internat. Contre le Cancer 15 (3-4), 715-722 (1959).

6. Vorwald, A. J., and Reeves, A. L.: An Equipped Horizontal Cold Cabinet. Chemist-Analyst 50 (3), 119-120 (1961).

7. Reeves, A. L., and Vorwald, A. J.: The Humoral Transport of Beryllium. J. Occup. Med. 3 (12), 567-574 (1961).

8. Reeves, A. L., Vorwald, A. J., Urban, E. C. J., and MacEwen, J. D.: The Kinetics of Pulmonary Deposition and Clearance of Inhaled Beryllium Sulfate in the Rat. Fed. Proc. 21 (2), 446 (1962).

9. Reeves, A. L.: The Absorption of Beryllium from the Gastrointestinal Tract. Arch. Environ. Health 11 (8), 209-214 (1965).

10. Vorwald, A. J., Reeves, A. L., and Urban, E. C. J.: Experimental Beryllium Toxicology. In: Beryllium--Its Industrial Hygiene Aspects (H. E. Stokinger, Editor). pp. 201-234. Academic Press, New York, 1966.

11. Reeves, A. L., Busby, E. K., and Scotti, L.: Gel Electrophoresis in the Study of Pneumoconioses. Amer. Indust. Hyg. Assoc. J. 27 (3), 278-287 (1966).

12. Reeves, A. L.: Lung and Lymph Node Isozymes During Beryllium-Induced Pulmonary Carcinogenesis. Proc. Amer. Assoc. Cancer Research 7 (1), (1966).

13. Reeves, A. L.: Pulmonary Isozyme Profiles During Experimental Beryllium Carcinogenesis. Proc. 13th Internat. Congress on Occup. Health 3, 133-136 (1966).

14. Reeves, A. L., Deitch, D., and Vorwald, A. J.: Beryllium Carcinogenesis. I. Inhalation Exposure of Rats to $BeSO_4$ Aerosol. Cancer Research 27 (I), 439-445 (1967).

15. Reeves, A. L., and Vorwald, A. J.: Beryllium Carcinogenesis. II. Pulmonary Deposition and Clearance of Inhaled $BeSO_4$ in the Rat. Cancer Research 27 (I), 446-451 (1967).

16. Reeves, A. L.: Isozymes of Lactate Dehydrogenase During Beryllium Carcinogenesis in the Rat. Cancer Research 27 (I), 1895-1899 (1967).

17. Reeves, A. L.: Über die Retention von Eingeatmetem Berylliumsulfat-Aerosol in Rattenlungen. Internat. Arch. Gewerbepath. Gewerbehyg. 24, 226-237 (1968).

18. Reeves, A. L.: Effect of Age on the Severity of Toxic Response. Amer. Indust. Hyg. Assoc. J. 30 (2), 110-111 (1969).

19. Davis, H. V., and Reeves, A. L.: Pulmonary Fibrogenesis in Rats Exposed to Asbestos. Amer. Indust. Hyg. Assoc. J. 30 (2), 156-157 (1969).

8000 1138

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002080

Publications Cont'd (Dr. A. L. Reeves)

20. Reeves, A. L., and Deitch, D.: Influence of Age on the Carcinogenic Response to Beryllium Inhalation. Proc. 16th Internat. Congress on Occup. Health F6-66, 651-652 (1969).

21. Reeves, E. H., Reeves, A. L., and Nedwicki, E. G.: Miliary Tuberculosis and Silicosis. Proc. 16th Internat. Congress on Occup. Health F6-81, 678-679 (1969).

22. Reeves, A. L., Swanborg, R. H., Busby, E. K., and Krivanek, N. D.: The Role of Immunologic Reactions in Pulmonary Berylliosis. In: Inhaled Particles III (W. H. Walton, Editor). pp. 599-608. Unwin Bros. Ltd., Old Woking, Surrey, 1971.

23. Davis, H. V., and Reeves, A. L.: Collagen Biosynthesis in Rat Lungs During Exposure to Asbestos. Amer. Indust. Hyg. Assoc. J. 32 (9), 599-602 (1971).

24. Reeves, A. L., Puro, H. E., Smith, R. G., and Vorwald, A. J.: Experimental Asbestos Carcinogenesis. Environ. Research 4 (6), 496-511 (1971).

25. Krivanek, N. D., and Reeves A. L.: Effects of Chemical Forms of Beryllium on the Production of Immunologic Response. Amer. Indust. Hyg. Assoc. J. 33 (1), 45-52 (1972).

26. Reeves, A. L.: The Effect of Air Pollution on Animal Health. In: The Air Pollution Manual 2nd Ed. (P. Giever, Editor). pp. 45-59. American Industrial Hygiene Association, Westmont, New Jersey, 1972.

27. Reeves, A. L., Puro, H. E., Smith, R. G., and Vorwald, A. J.: Fibrogenicity and Carcinogenicity of Amosite, Crocidolite, and Chrysotile in Animal Experiment. Amer. Indust. Hyg. Assoc. J. 33 (2), A79 (1972).

28. Reeves, A. L., Krivanek, N. D., Busby, N. K., and Swanborg, R. H.: Immunity to Pulmonary Berylliosis in Guinea Pigs. Internat. Arch. Occup. Health 29, 209-220 (1972).

29. Reeves, A. L., Puro, H. E., and Reeves, A. L.: Fibrogenesis and Carcinogenesis from Various Forms of Asbestos. Proc. 17th Internat. Congress on Occup. Health in press.

30. Reeves, A. L.: Cutaneous Hypersensitivity and Pulmonary Response to Beryllium. Proc. 17th Internat. Congress on Occup. Health in press.

31. Roy-Chowdhury, A. K., Mooney, T. F., and Reeves, A. L.: Trace Metals in Asbestos Carcinogenesis. Arch. Environ. Health in press.

32. Reeves, A. L., and Krivanek, N. D.: The Influence of Cutaneous Hypersensitivity to Beryllium on the Development of Experimental Pulmonary Berylliosis. Arch. Environ. Health in press.

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

8000 1139

HFM - 002081

SECTION II — PRIVILEGED COMMUNICATION

BIOGRAPHICAL SKETCH

(Give the following information for all professional personnel listed on page 3 beginning with the Principal Investigator. Use continuation pages and follow the same general format for each person.)

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Henry E. Puro	Associate Professor	10-7-1923
PLACE OF BIRTH (City, State, Country)	**PRESENT NATIONALITY (If not U.S. citizen, indicate kind of visa and expiration date)**	**SEX**
Evanston, Illinois	USA	☒ Male ☐ Female

EDUCATION *(Begin with baccalaureate training and include postdoctoral)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	SCIENTIFIC FIELD
Stanford University, Palo Alto, Calif.	A. B.	1946	Pre-medical
Stanford University, Palo Alto, Calif.	M. D.	1949	Medicine
Am. Board of Pathology, Tampa, Florida	Diplomate	1955	Pathologic anatomy

HONORS

Fellow, Royal Microscopical Society
Member, International Academy of Pathology
Member, College of American Pathologists

MAJOR RESEARCH INTEREST	ROLE IN PROPOSED PROJECT
Microscopic Pathology	Pathologist

RESEARCH SUPPORT *(See instructions)*

U. S. Public Health Service Grant 2-RO1-EC 00240, "Experimental Asbestos Carcinogenesis", 8-1-71 to 7-31-73, total support $121,492, current year $58,050, 25% of time.

RESEARCH AND/OR PROFESSIONAL EXPERIENCE *(Starting with present position, list training and experience relevant to area of project. List all or most representative publications. Do not exceed 3 pages for each individual.)*

1967-present Associate Professor, Department of Pathology, Wayne State University
1960-1967 Assistant Professor, Department of Pathology, Wayne State University
1956-1960 Pathologist, Lynn Hospital, Allen Park, Michigan
1954-1956 Instructor, Department of Pathology, Stanford University
1953-1954 Fellow in Pathology, Stanford University
1949-1950 Resident in Pathology, Stanford University
1948-1949 Intern, Highland-Alameda County Hospital

Publications:

1. Puro, H. E., and [illegible] Rhabdomyomatous Skeletal Muscle, Am. J. Surg. 89, 1187-1190 (1955).
2. Wendt, V. E., Puro, H. E., Shapiro, J., Mathews, W., and Wolf, P.: Angiothrombotic Pulmonary Hypertension in Addicts ("Blue Velvet" addiction), J. Amer. Med. Assoc. 188, 755-757 (1964).
3. Puro, H. E., Wolf, P. L., Long, R., and Vazquez, J.: Experimental Production of Pulmonary Vasculitis Utilizing Paregoric Pyribenzamine (Blue Velvet) in Rabbits, Amer. J. Path. 46, 44A (1965).

(Cont'd)

8000 1140

U. S. GOVERNMENT PRINTING OFFICE : 1971 O - 451-738

HFM - 002082

Publications Cont'd (Dr. H. E. Puro)

4. Puro, H. E., Epstein, E., and Zak, B.: Adaption of Manual Fluorometer for Continuous Paper Strip Scanning. Chemist-Analyst 54, 2 (1965).

5. Keech, M. D., and Puro, H. E.: Fatal Polyarteritis Presenting as Asymmetrical Peripheral Gangrene. Ann. Rheum. Dis. 24, 549-557 (1965).

6. Puro, H. E., Wolf, P. L., Skirgaudas, J., and Vazquez, J.: Experimental Production of Human "Blue Velvet" and "Red Devil" Lesions in Rabbits. J. Amer. Med. Assoc. 197, 1100-1102 (1966).

7. Bianchi, F. A., Bistue, A. R., Wendt, V. E., Puro, H. E., and Keech, M. K.: Analysis of Twenty-Seven Cases of Progressive Systemic Sclerosis (Including Two with Combined Systemic Lupus Erythematosus) and a Review of the Literature. J. Chron. Dis. 19, 953-977 (1966).

8. Wilson, R. F., Chen, S. C., and Puro, H. E.: Preoperative Irradiation of Cancer of the Lung. Cancer 23, 419-430 (1969).

9. Thomas, N. W., H. E. Puro, and Arbulu, A.: Significance of Hemoptysis in Lung Abscess. J. Thor. Cardiovasc. Surg. 59, 617-629 (1970).

10. Reeves, A. L., Puro, H. E., Smith, R. G., and Vorwald, A. J.: Experimental Asbestos Carcinogenesis. Environ. Research 4 (6), 496-511 (1971).

11. Reeves, A. L., Puro, H. E., Smith, R. G., and Vorwald, A. J.: Fibrogenicity and Carcinogenicity of Amosite, Crocidolite, and Chrysotile in Animal Experiments. Amer. Indust. Hyg. [illegible]

12. Reeves, A. L., Puro, H. E., and Reeves, E. H.: Fibrogenesis and Carcinogenesis from Various Forms of Asbestos. Proc. 17th Internat. Congress on Occup. Health in press.

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

8000 1141

HFM - 002083

SECTION II – PRIVILEGED COMMUNICATION

BIOGRAPHICAL SKETCH

(Give the following information for all professional personnel listed on page 3, beginning with the Principal Investigator. Use continuation pages and follow the same general format for each person.)

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Anil Kumar Roy-Chowdhury	Research Associate	7-24-1939
PLACE OF BIRTH (City, State, Country)	**PRESENT NATIONALITY (If non-U.S. citizen, indicate kind of visa and expiration date)**	**SEX**
Calcutta, India	India Permanent U.S. Resident, Reg. No. A18-972-616	☒ Male ☐ Female

EDUCATION *(Begin with baccalaureate training and include postdoctoral)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	SCIENTIFIC FIELD
Presidency College, Calcutta, India	B. S.	1960	Chemistry
University of Calcutta, Calcutta, India	M. S.	1962	Chemistry
University of Calcutta, Calcutta, India	Ph. D.	1966	Physical Chemis

HONORS

Fellow, Council of Scientific and Industrial Research, India.
Member, Chemical Society of India.
Member, American Chemical Society.

MAJOR RESEARCH INTEREST	ROLE IN PROPOSED PROJECT
Aerosol technology and chemistry	Chemist

RESEARCH SUPPORT *(See instructions)*

U.S. Public Health Service Grant 2-RO1-ES 00353, "The Pathogenesis of Pulmonary Beryllio[illegible] current year $[illegible] of time.

U.S. Public Health Service Grant 2-RO1-EC 00240, "Experimental Asbestos Carcinogenesis", 8-1-71 to 7-31-73, total support $121,492, current year $58,050, 75% of time

RESEARCH AND/OR PROFESSIONAL EXPERIENCE *(Starting with present position, list training and experience relevant to area of project. List all or most representative publications. Do not exceed 3 pages for each individual.)*

1971-present Research Associate, Dept. of Occup. Environ. Health, Wayne State U.
1969-1970 Post-Doctoral Fellow, University of Missouri at Rolla
1967-1969 Scientific Officer, Central Research Inst., Fertilizer Corp. of India, Ltd

Publications:

1. Pal, P. K., and Roy-Chowdhury, A. K.: On the Stability and Electrokinetic Potential of Cadmium Ferrocyanide Sol. J. Indian Chem. Soc. 41, 529-533 (1964).
2. Roy-Chowdhury, A. K., and Ghosh, B. N.: Studies on Adsorption of Cetyltrimethyl Ammonium Bromide on Xylene Globules Dispersed in Water. J. Indian Chem. Soc. [illegible]
3. Ghosh, B. N., Sengupta, S. K., and Roy-Chowdhury, A. K.: Effect of Temperature on the Rate of Coagulation of Thorium Oxide and Ceric Oxide Hydrosols. J. Indian Chem. Soc. 44, 1077-1081 (1967).
4. Mishra, M. B., Roy-Chowdhury, A. K., and Banerju, B. K.: Estimation of Phosphate--A Rapid Polarographic Method, Technology. Fertilizer Corp. Ind. J. 5, 307-310 (1968).
5. Roy-Chowdhury, A. K., Mooney, T. F., and Reeves, A. L.: Trace Metals in Asbestos Carcinogenesis. Arch. Environ. Health in press.

U. S. GOVERNMENT PRINTING OFFICE : 1971 O - 451-736

HFM - 002084

PART II — SUPPLEMENTARY INFORMATION

BIOGRAPHICAL SKETCH

(Give the following information for all professional personnel listed on page 3, beginning with the Principal Investigator. Use continuation pages and follow the same general format for each person.)

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Neil D. Krivanek	Instructor	6-11-1944
PLACE OF BIRTH (City, State, Country)	**PRESENT NATIONALITY (If non-U.S. citizen, indicate kind of visa and expiration date)**	**SEX**
Milwaukee, Wisconsin	USA	☒ Male ☐ Female

EDUCATION *(Begin with baccalaureate training and include postdoctoral)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	SCIENTIFIC FIELD
University of Wisconsin, Madison, Wisc.	B. S.	1966	Chemistry
Wayne State University, Detroit, Mich.	M. S.	1968	Biochemistry
Wayne State University, Detroit, Mich.	M. S.	1970	Industrial Hygiene
Wayne State University, Detroit, Mich.	Ph. D.	1972	Physiol. & Pharm.

HONORS

Public Health Service Fellow, Wayne State University
Member, American Chemical Society

MAJOR RESEARCH INTEREST	ROLE IN PROPOSED PROJECT
Immunobiology, Toxicology	Assistant Principal Investigator

RESEARCH SUPPORT *(See instructions)*

None

RESEARCH AND/OR PROFESSIONAL EXPERIENCE *(Starting with present position, list training and experience relevant to area of project. List all or most representative publications. Do not exceed 3 pages for each individual.)*

1972-present Instructor, Dept. of Occup. Environ. Health, Wayne State U.
1968-1972 Public Health Service Trainee, Dept. of Occup. Environ. Health, Wayne State U.

Publications:

1. Reeves, A. L., Swanborg, R. H., Busby, E. K., and Krivanek, N. D.: The Role of Immunologic Reactions in Pulmonary Berylliosis. In: Inhaled Particles III (W. H. Walton, Editor). pp. 599-608. Unwin Bros. Ltd., Old Woking, Surrey, 1971.

2. Krivanek, N. D., and Reeves, A. L.: Effects of Chemical Forms of Beryllium on the Production of Immunologic Response. Amer. Indust. Hyg. Assoc. J. 33(1), 45-52 (197

3. Reeves, A. L., Krivanek, N. D., Busby, E. K., and Swanborg, R. H.: Immunity to [illegible] Berylliosis in Guinea Pigs. Internat. Arch. Occup. Health 29: 209-220

4. Reeves, A. L., and Krivanek, N. D.: The Influence of Cutaneous Hypersensitivity to Beryllium on the Development of Experimental Pulmonary Berylliosis. Arch. Environ. Health in press.

8000 1143

U. S. GOVERNMENT PRINTING OFFICE : 1971 O - 451-730

HFM - 002085

RESEARCH PLAN

A. Introduction

1. Objective

The long-term goal of this research is to understand the carcinogenic activity of asbestos in terms of the etiologic factor(s) inherent in or associated with the fibers. Studies are currently in progress to modify fibers and test their remaining carcinogenic potency in experimental animals. The objective of this application is to continue with these studies and to establish circumstances or devise means by which exposures could be rendered harmless.

2. Background

The carcinogenic potential of asbestos in the environment has become one of the leading public health concerns of our time. The hazards of asbestos exposure were well demonstrated in miners and mill workers (46), pipe insulation workers (66), shipyard workers (36), coke oven operators (64), cigarette filter manufacturers (24), and automobile brake repairmen (37). Asbestos hazard was also considered to originate from battery boxes (26), protective clothing (2), and certain types of soils during agricultural work (10). Non-occupational and non-respiratory exposure to potentially significant quantities of asbestos was postulated for consumers of talc-coated rice in view of the tremolite asbestos in the talc (48); various bottled beverages including beer (14); and parenteral drugs (51). In the two latter cases, the asbestos content of the fluids was contributed from the filters during filtration, and it was speculated that migration of the fibers from the gastrointestinal tract to mesothelial tissue might occur in a similar way as from subcutaneous injection sites (42).

The presence of asbestos fibers in urban air is now well established (68), although whether or not the ambient concentration is high enough to constitute a hazard is uncertain (57). In the lung of city dwellers, both uncoated asbestos (43), and ferruginous bodies (30) are common findings, although the central core of the latter is not always asbestos (31). Much of the atmospheric asbestos in the cities probably originates from building demolition and weathering. The significance of [illegible] is uncertain, since at the temperature of brake application part or all of asbestos undergoes crystallographic transformation into nonfibrous pyroxenes and forsterite (70). However, the degree of completion of this transformation during actual vehicle operation is not known, nor has the chronic inhalation toxicity of the heat decomposition products of asbestos been thus far adequately investigated (41).

Malignant neoplasia now recognized to result from the inhalation of asbestos [illegible] of the lung and mesothelioma of the pleura and peritoneum (32). Also suspected are links to cancers of the gastrointestinal tract (63), hematopoietic system (33) and female reproductive system (27).

Experimental work in asbestos carcinogenesis was recently reviewed by this writer (55). Briefly, pleural and peritoneal mesotheliomas were readily produced through local implantation of asbestos fibers into various laboratory animals by Wagner (79), Donna (20), Stanton et al. (71), and others; a few lung cancers were also produced in rats by inhalation of chrysotile or crocidolite by Gross et al. (29)

8000 1144

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002086

and Reeves et al. (55). In the Progress Report attached to this application, we also report on the production of carcinoma of the lung and mesothelioma of the pleura in rats after amosite inhalation. Recently, Roe (59) has obtained pleural and peritoneal mesotheliomas in mice after subcutaneous injection of amosite and crocidolite in the crude form but not after solvent extraction; Stanton and Wrench (72) have produced mesotheliomas after pleural implantation of not only amosite, crocidolite, and chrysotile, but also of fibrous glass. These experiments raise the question of the carcinogenic entity present in asbestos, and its relative efficacy among the several mineralogical species.

Until recently, this writer (56) as well as Wagner and Berry (81) believed, on hand of preliminary experience, that amosite was less carcinogenic than other asbestiform minerals. It is now clear that this belief must be modified; recent research in this laboratory and elsewhere has shown unequivocally that amosite possesses distinct carcinogenic potential in experimental animals (59, 72, see also Progress Report, p. 27) as well as in man (67). There appears to be adequate evidence now to indicate that all other parameters being equal, no essential difference exists between the major varieties of asbestos with respect to their carcinogenic capacities, even in cases where their fibrogenic efficiencies might be different.

The question of critical particle size for maximum carcinogenic activity is not completely resolved. Thus far, the majority of investigations and speculations in that regard referred to fibrogenesis, and a lower threshold of fiber length of 2-10 μ (39, 80, see also Progress Report, p. 30) was suggested below which the fibrogenic potential rapidly diminished, presumably because of the easier lymphogenic transportability of the dusts (3, 38). Current lower threshold for asbestos fiber length in the ACGIH-TLV list is at 5 μ (73). Stanton and Wrench (72) obtained a lower tumor yield with excessively milled and partially pulverized crocidolite where most particles did not exceed 2-3 μ in their diameter, than with conventionally milled crocidolite containing a substantial component of longer fibers. Bryson and Bischoff (9) suggested that the carcinogenicity of asbestos may not be a primary property of the fibers but is perhaps attributable to reaction to the avascular fibrous tissue formed in response to the fibrogenic action of the dusts. This opinion assumes an intimate interdependence between the fibrogenic and carcinogenic capacities of asbestos. However, such interdependence does not seem to be proven thus far (56) and it is apparent that a carcinogenic stimulus might result in a secondary location such as the pleura or peritoneum, if translocated fibers become lodged there in the course of lymphogenic clearance from the primary site of deposition. Even though fibrous pleural plaques are common findings in asbestos-exposed humans (3, 76), it is not at all certain that tumor formation at a site is dependent on prior fibrotic reaction on that site. In our recent studies summarized in the attached Progress Report, chrysotile inhalation with a light microscopic fiber count of 54/ml and median electron microscopic particle size of 0, 2 X 6-15 μ, caused only slight degree of asbestosis but substantial pulmonary tumor incidence.

Essentially, the carcinogenic entity present in asbestos may be either physical or chemical. The physical hypothesis, according to which the mechanical irritation attributable to the embedded fibers or to the ensuing biological response (Oppenheimer effect) is the key factor in the etiology of asbestos cancers was not viewed with favor by the early investigators (33) but received new support from the

8000 F145

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002087

studies of Stanton and Wrench, who obtained mesotheliomas with fibrous glass if the latter was milled to approach the size range of carcinogenic asbestos fibers (72). These authors therefore concluded that carcinogenicity was primarily related to the structural shape of these materials rather than to physicochemical properties.

The chemical hypothesis is espoused by numerous other authors who believe that a chemical or physicochemical factor, associated either with the silicate core of asbestos fibers or with certain adventitious factors, is the ultimate carcinogenic principle. Thus far, the question of adventitious factors has received the most attention. Harington (32) discovered that certain virgin samples of asbestos contained cyclohexane-extractable oils composed of aromatic hydrocarbons, one of which was 3,4-benzpyrene. Furthermore, it was pointed out that besides natural trace constituents which apparently became adsorbed on asbestos fibers during their geological genesis, additional opportunities for contamination exist during commercial handling. Jute bags (60) as well as polythene bags (11) were shown to release aromatic compounds of possible or proven carcinogenic potential. According to one set of authors, the removal of these contaminants somewhat reduced but did not eliminate the carcinogenic activity of asbestos (35), and addition of 3,4-benzpyrene to chrysotile did not increase its carcinogenic potential (54). According to another set of authors, solvent extraction of asbestos samples has completely eliminated the causation of mesotheliomas distant to the subcutaneous injection site (59), and addition of 3,4-benzpyrene to chrysotile distinctly increased its carcinogenic potential (61). The question of these contradictory results is at present not resolved.

Metals present in asbestos include iron, aluminum, and magnesium as major constituents and chromium, nickel, cobalt, manganese, and others as trace constituents. Several of the latter are known respiratory carcinogens although it was argued (72) that their levels in asbestos are too small to be biologically significant. However, it was pointed out by Cralley et al. (13) that during manufacture of asbestos textile products the concentration of carcinogenic metals in asbestos increases substantially through contact with the weaving machinery. Similar increase in heav metal content of asbestos was also experienced during chamber dissemination as practiced by Gross et al. (29) and Reeves et al. (55, see also Progress Report, p. [illegible]). Dixon et al. (19) have attempted to explain the carcinogenic activity of asbestos-associated trace metals on the grounds of inhibition of benzpyrene hydroxylase, a detoxifying enzyme that presumably protects the organism from the effects of benzpyrene. Cralley (12) suggested that adsorbed metal on asbestos fibers may become the poles of miniature electrolytic cells, and the ensuing electromotive forces may produce high levels of biologically active cations at localized tissue sites. These interesting hypotheses are not proven at this time.

Equally interesting is the question of host susceptibility to asbestos cancers. The epidemiological finding that cigarette smokers have a substantially increased hazard in this respect (5, 65, 66) is probably connected with adsorptive phenomena, but animal experiments have shown that widely different responsiveness exists among various species of rodents to asbestos (15, 28, 80, see also Progress Report p. 29). In some cases this was attributable to relative efficiencies of pulmonary clearance, and the unsuitability of hamsters to develop cancers from asbestos is apparently due to a swift and severe fibrotic response which curtails the life

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

8000 1146 PQ

HFM - 002088

expectancy of the exposed animals. However, in two experiments performed at this laboratory (55, 56, see also Progress Report, p. 29), guinea pigs showed adequate survival and no carcinogenic response after either asbestos implantation or inhalation. Gerbils showed no carcinogenic response after inhalation, and rabbits developed pleural mesotheliomas after direct implantation only. Without question, the most suitable experimental animal species for asbestos cancer production is the rat, and the reasons for this apparent species specificity are entirely unknown However, it is remarkable that after equal asbestos exposure, pulmonary ferruginous bodies are abundant in the guinea pig and rare in the rat.

There is now growing evidence that the formation of ferruginous bodies is the result of a phagocytic process involving several macrophages at a site, which fuse to form giant cells (16, 17, 74). This process results in the coating of fibrous particles with a mucopolysaccharide layer containing hemosiderin granules (25), irrespective of whether the phagocytized fiber is asbestos, fibrous glass, or other ceramic silicate (7, 18). Ferruginous body formation is thus apparently a protective reaction of the organism. In rodents, there appears to be an inverse relation between the frequency of these bodies and the carcinogenic effect attributable to inhaled asbestos. The existence of an immunologic factor in the causation of asbestosis or asbestos neoplasia has been considered (47) but serum immunoelectrophoretic studies in asbestos cement workers (21) and pulmonary function studies in asbestos-exposed rabbits with and without administration of the immunosuppressive drug Imuran (brand of Azathioprine, Burroughs Wellcome & Co.) were thus far inconclusive (22). A study attempting to correlate ferruginous body count to degree of pathology in the lungs of laboratory animals is currently in progress at our laboratory (cf. Progress Report, p. 31).

The *in vitro* action of asbestos on various model systems including tissue culture was studied as a possible adjunct to the understanding of its fibrogenic and carcinogenic properties. Pernis and Gastano (53) have found no cytotoxicity for chrysotile or crocidolite to mouse peritoneal macrophages under ideal culture conditions, and no interruption of cellular lactate biosynthesis was observed (4). However, membrane permeability as measured by eosin uptake was increased after contact with asbestos, which could lead to lysis of the cells in the absence of protective factors (1). Glass fiber, but not glass powder, was shown to have similar effects (78). It was also observed that passage through asbestos filters conferred a growth-inhibitory property on the medium used in the culture of HeLa cells (44). Bey and Harington (6), Miller and Harington (49), as well as Robock and Klosterkötter (58) have found higher general cytotoxicity for chrysotile than for the amphiboles, and chrysotile, but not amosite or crocidolite, was found to be a powerful hemolytic agent (34, 45, 62). That amphibole dusts (amosite and crocidolite) should nonetheless appear to be more hazardous upon inhalation than chrysotile was explainable on the grounds that the shorter and harsher fibrils of the former have a better opportunity to penetrate into the alveoli or pleura while chrysotile, in view of its curved shape, is more likely to get arrested high in the respiratory tract (58

The *in vivo* solubility of chrysotile was investigated with radioactive tracer techniques (78) by Morgan *et al.*, detecting migration of certain fractions to the live (50). Special vulnerability of the pleural mesothelium by chrysotile was shown by measuring the turnover of tritiated thymidine (8); collagen biosynthesis as

☆U.S.GPO: 1970 - 379-[illegible]

8000 1147

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002089

measured by rate of proline hydroxylation was highest in the early stages of tissue contact with asbestos (15).

The toxicity of modified asbestos fibers was investigated only very incompletely thus far. Beck et al. (4) have treated chrysotile with 0.1 N HCl, leaving a SiO_2 surface on the fibers, and observed increased acute cytotoxicity as manifested by reduced lactate biosynthesis. Heating an impure specimen of chrysotile (fibrous component about 50%, the rest mainly antigorite) to 1000° C for 3 hours caused greatly increased acute toxicity after intraperitoneal injection into mice (41). The heat treatment caused appearance of forsterite and enstatite detectable by x-ray diffraction, but the acute toxicity may have been due to a volatile component.

Trace metal removal experiments from asbestos fibers are currently in progress at our laboratory. These experiments involve dilute acid treatment, chelation with EDTA, and ionic exchange. The modified specimens were examined by atomic absorption spectrophotometry and x-ray diffraction, and are currently tested for carcinogenicity by the intraperitoneal injection method (cf. Progress Report, p. 31).

References:

1. Allison, A. C.: Effects of Silica and Asbestos on Cells in Culture. In: Inhaled Particles III (W. H. Walton, Editor). pp. 437-445. Unwin Bros. Ltd., Old Woking, Surrey, 1971.
2. Bamber, H. A., and Butterworth, R.: Asbestos Hazard from Protective Clothing. Ann. Occup. Hyg. 13, 77-79 (1970).
3. Beck, E. G., Bruch, J., Friedrichs, K. H., Hilscher, W., and Pott, F.: Fibrous Silicates in Animal Experiments and Cell Culture--Morphological Cell and Tissue Reactions According to Different Physical Chemical Influences. In: Inhaled Particles III (W. H. Walton, Editor). pp. 477-487. Unwin Bros. Ltd., Old Woking, Surrey, 1971.
4. Beck, E. G., Holt, P. F., and Nasrakah, E. T.: Effects of Chrysotile and Acid-Treated Chrysotile on Macrophage Cultures. Brit. J. Indust. Med. 28, 179-185 (1971).
5. Berry, G., Newhouse, M. L., and Turok, M.: Combined Effects of Asbestos Exposure and Smoking on Mortality from Lung Cancer in Factory Workers. Lancet ii, 476-478 (1972).
6. Bey, E., and Harington, J. S.: Cytotoxic Effects of Some Mineral Dusts on [illegible] Exp. Med. 133, 1149-1169 (1971).
7. Botham, S. K., and Holt, P. F.: The Development of Glass-Fibre Bodies in the Lungs of Guinea Pigs. J. Path. 103, 149-156 (1971).
8. Bryks, S., and Bertalanffy, F. D.: Cytodynamic Reactivity of the Mesothelium--Pleural Reaction to Chrysotile Asbestos. Arch. Environ. Health 23, 469-472 (1971).
9. Bryson, G., and Bischoff, F.: Silicate-Induced Neoplasms. Progr. Exp. Tumor Research 9, 77-164 (1967).

U.S.GPO: 1970 - 370-[illegible]

8000 1148 PQ

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002090

10. Burilkov, T., and Michailova, L.: Asbestos Content of the Soil and Endemic Pleural Asbestosis. Environ. Research 3, 443-451 (1970).

11. Commins, B. T., and Gibbs, G. W.: Contaminating Organic Material in Asbestos. Brit. J. Cancer 23, 358-362 (1969).

12. Cralley, L. J.: Electromotive Phenomenon in Metal and Mineral Particulate Exposures: Relevance to Exposure to Asbestos and Occurrence of Cancer. Am. Indust. Hyg. Assoc. J. 32, 653-661 (1971).

13. Cralley, L. J., Keenan, R. G., and Lynch, J. R.: Exposure to Metals in the Manufacture of Asbestos Textile Products. Am. Indust. Hyg. Assoc. J. 28, 452-461 (1967).

14. Cunningham, H. M., and Pontefract, R.: Asbestos Fibers in Beverages and Drinking Water. Nature 232, 332-333 (1971).

15. Davis, H. V., and Reeves, A. L.: Collagen Biosynthesis in Rat Lungs During Exposure to Asbestos. Am. Indust. Hyg. Assoc. J. 32, 599-602 (1971).

16. Davis, J. M. G.: Asbestos Dust as a Nucleation Center in the Calcification of Old Fibrous Tissue Lesions, and the Possible Association of this Process to the Formation of Asbestos Bodies. Exp. & Mol. Pathol. 12, 133-147 (1970).

17. Davis, J. M. G.: Further Observations on the Ultrastructure and Chemistry of the Formation of Asbestos Bodies. Exp. & Mol. Pathol. 13, 346-358 (1970).

18. Davis, J. M. G., Gross, P., and DeTreville, R. T. P.: Ferruginous Bodies in Guinea Pigs. Arch. Path. 89, 364-373 (1970).

19. Dixon, J. R., Lowe, D. B., Richards, D. E., Cralley, L. J., and Stokinger, H. E.: The Role of Trace Metals in Asbestos Carcinogenesis: Asbestos Cancers. Cancer Research 30, 1068-1074 (1970).

20. Donna, A.: Tumori Sperimentali da Amianto di Crisotilo, Crocidolite, e Amosite in Ratto. Med. Lavoro 61, 1-32 (1970).

21. El-Sewely, A. Z., and Hassan, [illegible]: Immunoelectrophoretic Pattern Changes in Asbestosis. Ann. Occup. Hyg. 14, 25-28 (1971).

22. Ford, J. E.: The Effect of Pulmonary Macrophage Suppression in Developing Asbestosis. Dissertation, Wayne State University, Detroit, 1971.

23. Gerber, M. A.: Asbestosis and Neoplastic Disorders of the Hematopoietic System. Am. J. Clin. Path. 53, 204-208 (1970).

[illegible] Goff, A. M., and Gaensler, E. A.: Asbestosis Following Brief Exposure in Cigarette Filter Manufacture. Respiration 29, 83-93 (1972).

[illegible] Governa, M., and Rosanda, [illegible]: A Histochemical Study of the Asbestos Body Coating. Brit. J. Indust. Med. 29, 154-159 (1972).

26. Greenberg, M.: Asbestos Release from Battery Boxes. Ann. Occup. Hyg. 13, 79-80 (1970).

27. Graham, J., and Graham, R.: Ovarian Cancer and Asbestos. Environ. Research 1, 115-128 (1967).

☆U.S.GPO: 1970 - 379-565

8000 1149 PQ

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002091

28. Gross, P., and DeTreville, R. T. P.: Experimental Asbestosis. Arch. Environ. Health 15, 638-649 (1967).

29. Gross, P., DeTreville, R. T. P., Tolker, E. B., Kaschak, M., and Babyak, M. A.: Experimental Asbestosis--The Development of Lung Cancer in Rats with Pulmonary Deposits of Chrysotile Dust. Arch. Environ. Health 15, 343-355 (1967).

30. Gross, P., DeTreville, R. T. P., and Haller, M. N.: Pulmonary Ferruginous Bodies in City Dwellers: A Study of Their Central Fiber. Arch. Environ. Health 19: 186-188 (1969).

31. Gross, P., Tuma, J., and DeTreville, R. T. P.: Fibrous Dust Particles and Ferruginous Bodies. Arch. Environ. Health 21, 38-46 (1970).

32. Harington, J. S.: Occurrence of Oils Containing 3,4-Benzpyrene and Related Substances in Asbestos. Nature 193, 43-45 (1962).

33. Harington, J. S.: Studies of Carcinogenesis of Asbestos Fibers and Their Natural Oils. Ann. N. Y. Acad. Sci. 132, 439-50 (1965).

34. Harington, J. S., Miller, K., and MacNab, G.: Hemolysis by Asbestos. Environ. Research 4, 95-117 (1971).

35. Harington, J. S., Roe, F. J. C., and Walters, M.: Studies on the Mode of Action of Asbestos as a Carcinogen. S. Afr. Med. J. 41, 800-804 (1967).

36. Harries, P. G., MacKenzie, F. A. F., Sheers, G., Kemp, J. H., Oliver, T. P., and Wright, D. S.: Radiological Survey of Men Exposed to Asbestos in Naval Dockyards. Brit. J. Indust. Med. 24, 210 (1972).

37. Hickish, E. E., and Knight, K. L.: Exposure to Asbestos During Brake Maintenance. Ann. Occup. Hyg. 13, 17-21 (1970).

38. Hilscher, W., Sethi, S., Friedrichs, K. H., and Pott, F.: Zusammenhänge zwischen Asbestose und Faserlänge. Naturwiss. 57, 356-357 (1970).

39. Holt, P. F., Mills, J., and Young, D. K.: Experimental Asbestosis with Four Types of Fibers. Ann. [illegible]

40. Hounam, R. F.: The Konimiser--A Dispenser for the Continuous Generation of Dust Clouds From Miligram Quantities of Asbestos. Ann. Occup. Hyg. 14, 329-335 (1971).

41. Jagatic, J., Rubnitz, M. E., Godwin, M. C., and Weiskopf, R. W.: Tissue Response to Intraperitoneal Asbestos with Preliminary Report of Acute Toxi- [illegible] Treated Asbestos in Mice. [illegible] 230 (1971).

42. Kanazawa, K., Birbeck, M. S. C., Carter, R. L., and Roe, F. J. C.: Migration of Asbestos Fibers from Subcutaneous Injection Sites in Mice. Brit. J. Cancer 24, 96-106 (1970).

43. Langer, A. M., Selikoff, I. J., and Sastre, A.: Chrysotile Asbestos in the Lungs of Persons in New York City. Arch. Environ. Health 22, 348-361 (1971).

44. Litterst, C. L., and Lichtenstein, E. P.: Toxicity of HeLa Cell Growth Medium After Passage Through Asbestos Filters. Lab. Pract. 19, 1221-1223 (1970).

☆U.S.GPO: 1970 - 376-666

8000 1150 PQ

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002092

45. MacNab, G., and Harington, J. S.: Haemolytic Activity of Asbestos and Other Mineral Dusts. Nature 214, 522-523 (1967).

46. McDonald, J. C., McDonald, A. D., Gibbs, G. W., Siemiatycki, J., and Rossiter, C. E.: Mortality in the Chrysotile Asbestos Mines and Mills of Quebec. Arch. Environ. Health 22, 677-687 (1971).

47. Massey, D., Lemonde, J., and Fournier-Massey, G.: Les Facteurs Immunologiques dans l' Amiantose. Union Med. Can. 100, 1588-1591 (1971).

48. Merliss, R. R.: Talc-Treated Rice and Japanese Stomach Cancer. Science 173, 1141-1142 (1971).

49. Miller, K., and Harington, J. S.: Some Biochemical Effects of Asbestos on Macrophages. Brit. J. Exp. Path. 53, 397-405 (1972).

50. Morgan, A., Holmes, A., and Gold, C.: Studies of the Solubility of Constituents of Chrysotile Asbestos in Vivo Using Radioactive Tracer Techniques. Environ. Research 4, 558-570 (1971).

51. Nicholson, W. J., Maggiore, C. J., and Selikoff, I. J.: Asbestos Contamination of Parenteral Drugs. Science 177, 171-173 (1972).

52. Occella, E., and Maddalon, G.: X-ray Diffraction Characteristics of Some Types of Asbestos in Relation to Different Techniques of Comminution. Med. Lavoro 54, 628-636 (1963).

53. Pernis, B., and Castano, P.: Effect of Asbestos on Cells in Vitro. Med. Lavoro 62, 120-129 (1971).

54. Pott, F., Huth, F., and Friedrichs, K. H.: Tumors of Rats After i. p. Injection of Powdered Chrysotile and Benzo(a)-pyrene. Zbl. Bakt. Hyg. B 155, 463-469 (1972).

55. Reeves, A. L., Puro, H. E., Smith, R. G., and Vorwald, A. J.: Experimental Asbestos Carcinogenesis. [illegible] (1971).

56. Reeves, A. L., Puro, H. E., Smith, R. G., and Vorwald, A. J.: Fibrogenicity and Carcinogenicity of Amosite, Crocidolite, and Chrysotile in Animal Experiment. Am. Indust. Hyg. Assoc. J. 33 (2), A 79 (1972).

57. Rickards, A. L., and Badami, D. V.: Chrysotile Asbestos in Urban Air. Nature 234, 93-94 (1971).

58. Robock, K., and Klosterkötter, W.: Biological Action of Different Asbestos Dusts with Special Respect to Fibre Length and Semiconductor Properties. In: Inhaled Particles III (W. H. Walton, Editor). pp. 465-475. Unwin Bros. Ltd., Old Woking, Surrey, 1971.

59. Roe, F. J. C.: Experimental Asbestos Carcinogenesis. Food & Cosmetics Toxicol. 6, 566-568 (1968).

60. Roe, F. J. C., Walters, M. A., and Harington, J. S.: Tumor Initiation by Natural and Contaminating Asbestos Oils. Internat. J. Cancer 1, 491-495 (1966).

PHS-398 (REV. 6 64)

Page 19

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

8000 1151 PQ

HFM - 002093

61. Rylev, L. N.: Morphological Lesions in Rat Lungs Induced by Intratracheal Injection of Pure Chrysotile Asbestos and a Chrysotile Asbestos-Benzo(a)-pyrene Mixture. Vop. Oncol. 18, 40-45 (1972).

62. Schnitzer, R. J., and Pundsack, F. L.: Asbestos Hemolysis. Environ. Research 3, 1-13 (1970).

63. Selikoff, I. J., Bader, R. A., Bader, M. E., Churg, J., and Hammond, E. C. Asbestos and Neoplasia. Am. J. Med. 42, 487-496 (1967).

64. Selikoff, I. J., and Hammond, E. C.: Asbestos Exposure to Coke Oven Operators. J. Occup. Med. 13, 496-497 (1971).

65. Selikoff, I. J., Hammond, E. J., and Churg, J.: Asbestos Exposure, Smoking, and Neoplasia. J. Am. Med. Assoc. 204, 104-110 (1968).

66. Selikoff, I. J., Hammond, E. C., and Churg, J.: Mortality Experiences of Asbestos Insulation Workers. In: Proc. Internat. Conf. of Pneumoconiosis (H. A. Shapiro, Editor) pp. 180-186. Oxford Univ. Press, Cape Town, 1970.

67. Selikoff, I. J., Hammond, E. C., and Churg, J.: Carcinogenicity of Amosite Asbestos. Arch. Environ. Health 35, 183-186 (1972).

68. Selikoff, I. J., Nicholson, W. J., and Langer, A. M.: Asbestos Air Pollution. Arch. Environ. Health 25, 1-13 (1972).

69. Smith, M. S., and Naylor, B.: A Method for Extracting Ferruginous Bodies from Sputum and Pulmonary Tissue. Am. J. Clin. Path. 58, 250-254 (1972).

70. Speil, S., and Leineweber, J. P.: Asbestos Minerals in Modern Technology. Environ. Research 2, 166-208 (1969).

71. Stanton, M. F., Blackwell, R., and Miller, E.: Experimental Pulmonary Carcinogenesis with Asbestos. Am. Indust. Hyg. Assoc. J. 30, 236-244 (1969).

72. Stanton, M. F., and Wrench, C.: Mechanisms of Mesothelioma Induction with Asbestos and Fibrous Glass. J. Nat. Cancer Inst. 48, 797-821 (1972).

73. Stokinger, H. E. (Chairman): Threshold Limit Values for Chemical Substances in Workroom Air. Am. Conf. Govt. Indust. Hygienists, Cincinnati, 1972.

[redacted] Study with Three Types of Asbestos. Environ. Research [redacted]

75. Timbrell, V.: Characteristics of the International Union Against Cancer [redacted] Preparations (H. A. Shapiro, Editor) [redacted] Oxford Univ. Press, Cape Town, 1970.

[redacted] Timbrell, V., Gilson, J. C., and Webster, I.: UICC Standard Reference Samples of Asbestos. Internat. J. Cancer 3, 406-408 (1968).

77. Timbrell, V., Hyett, A. W., and Skidmore, J. W.: A Simple Dispenser for Generating Dust Clouds from Standard Reference Samples of Asbestos. Ann. Occup. Hyg. 11, 273-281 (1968).

8080 1152 PQ

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002094

78. Turnock, A. C., Bryks, S., and Bertalanffy, F. D.: The Synthesis of Tritium-Labeled Asbestos for Use in Biological Research. Environ. Research 4, 86-94 (1971).

79. Wagner, J. C.: Experimental Production of Mesothelial Tumors of the Pleura by Implantation of Dusts in Laboratory Animals. Nature 196, 180-181 (1962).

80. Wagner, J. C.: Asbestosis in Experimental Animals. Brit. J. Indust. Med. 20, 1-12 (1963).

81. Wagner, J. C., and Berry, G.: Mesotheliomas in Rats Following Inoculation with Asbestos. Brit. J. Cancer 23, 567-581 (1969).

82. Wagner, J. C., Gilson, J. C., Berry, G., and Timbrell, V.: Epidemiology of Asbestos Cancers. Brit. Med. Bull. 27, 71-76 (1971).

3. Rationale

The foregoing review attempted to summarize the great strides which were made in asbestos cancer research during the recent past but also pointed out the areas of contradiction and uncertainty. Identity of the carcinogenic factor(s) present in asbestos fibers is still unknown. Solvent extraction experiments for the purpose of removing trace organic carcinogens have given contradictory results (cf. Background References 35, 59), and trace metal removal experiments are still in progress (cf. Progress Report, p. 32). Recent work (72) showing carcinogenic capacity for finely ground glass fibers has revived the question of whether the essential stimulus is chemical or physical. Coated fibers (ferruginous bodies) seem to appear with much greater frequency in those species which do not develop cancer from asbestos; precise correlative studies are in progress (p. 31). Almost nothing is known on the toxicology of heat decomposition products of asbestos even though this question has great significance in assessing the potential public health hazard originating from automotive brake emissions.

We propose to continue with our studies designed to answer these questions. We regard the intraperitoneal injection technique a suitable first assay method for the biological testing of dusts, but the ultimate confirmation of carcinogenicity, or lack of it, must come from inhalation experiments because particle size, shape, and aggregation properties influence the pulmonary deposition and clearance patterns of the dusts. Our laboratory is equipped to perform both types of studies, and contrary to preliminary indications based on the injection technique (55, 56) we have recently shown experimentally the inhalation carcinogenicity of amosite dust (p. 27). We propose to investigate the carcinogenic hazards arising from asbestos-containing commercial products under simulated use conditions. We shall expand our experimentation on the removal of potentially carcinogenic trace constituents from asbestos, attempt to devise a dissemination system free from contaminant contribution, and test the modified dusts in animal experiment. We also propose a study involving maintenance of continuous unilateral pneumothorax in mini-pigs which have previously received bilateral intrapleural asbestos deposits, in order to test the significance of mechanical irritation due to respiratory movements in asbestos carcinogenesis.

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002095

4. Comprehensive Prog

"Expe inogenesis"
C 40-06/07

January 1, 1 mber 31, 1972

B. Summary

Rats, rabbits, guinea pigs, gerbils, and mice were exposed to the inhalation of amosite, crocidolite, and chrysotile, 16 hrs/week, for 104 weeks. The asbestos specimens were ballmilled and disseminated through hammer mill and fan systems into the chambers. Atmospheric concentration was 47.9-50.2 mg/m^3, but only a fraction of the dust had retained its fibrous form through the dissemination procedure. Light microscopic fiber counts in chamber air were 864-1105/ml for amosite and crocidolite but only 54/ml for chrysotile. Chemical analysis showed substantial enrichment of the dusts (3x-9x) in chromium and nickel during ballmilling. All animals showed signs of asbestosis in proportion to their exposure and in addition there was the following incidence of malignant neoplasia: In the amosite group, 1 papillary carcin- [illegible] and [illegible] of the pleura; in the crocidolite group, adenocarcinoma and 3 squamous carcinomas of the lung; and in the chrysotile group, 1 papillary and [illegible] carcinoma of the lung and 1 fibrous tumor of the mediastinum. All these neoplasms were in rats while the other species did not develop neoplasia. Ferruginous bodies were conspicuous only in guinea pigs; gerbils developed frequent alveolar proteinosis. Trace metal removal experiments have yielded asbestos specimens with substantial reduction of chromium and nickel content. These are currently tested for carcinogenic activity through intraperitoneal injection into rats.

8000 1154

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002096

C. Detailed Report

1. Chamber Maintenance and Monitoring

Three "walk-in chambers" (12 x 12 ft. rooms with perforated walls) were utilized in this experiment, one each for the dissemination of amosite, crocidolite, and chrysotile. These were described in more detail previously (cf. Background, references 55 and 56). The dusts were blown into these chambers through a network of ducts with the aid of hammer mills and fan systems. The chambers were in operation 4 hrs/day, 4 days/week, with each Friday reserved as clean-up day. The exposure lasted 2 years (December 1969-December 1971) with 1480 cumulative total exposure hours.

Dust concentration was monitored regularly throughout the experiment, through weekly air samples collected in each of the chambers on millipore filters. The measurements yielded an overall mean (in mg/m^3, $\pm$ S. D.) of 48.6 $\pm$ 3.0 for amosite; 50.2 $\pm$ 3.6 for crocidolite; and 47.9 $\pm$ 3.0 for chrysotile. The aerosols appeared as dense dust clouds readily visible to the naked eye but with little tendency to settle on surfaces. The used asbestos was exhausted from each chamber into plastic collection bags.

2. Dust Characterization

Amosite (W-3 fibers), crocidolite (S-blue fibers) and chrysotile (3-T fibers) were obtained in repeated shipments from the Johns-Manville Corporation. The crude samples were ballmilled in glazed ceramic jars with stainless steel balls for 10 days, and the resultant substances forced through a #8 mesh screen for the disintegration of larger clumps. These dusts were fed into the hopper of the hammer mills and blown into the exposure chambers.

The milling procedure caused not only substantial reduction of particle size which was intended, but also a loss of fibrous structure in a great proportion of particles which was not intended. By using an axis-diameter ratio of 3:1 or more to define a fiber, it was found that only [illegible] dust collected on millipore filters in the chambers answered this definition during light microscopic examination. The upper limit of fiber length observable under the light microscope was 55-130 μ (comprising 0.001-0.015% of the dust) in various samples, and the fiber count per ml air was 864 (amosite), 1105 (crocidolite), or 54 (chrysotile). It is thus apparent that in the light microscopic size range the great majority of fibers were destroyed or transformed to an apparently nonfibrous crystal form. Similar results were obtained after a grinding procedure involving cutting (Timby, Oswalt and Maddalon (cf. Background, Reference 52), using x-ray crystallographic technique. It is well known that chrysotile is changed into forsterite at 810° C, and the amphiboles into pyroxenes at 900° C. Whether or not such high impact temperatures might have occurred during ball milling is not known. If they have, this might be a plausible explanation for the loss of fibrous structure in 98-99% of the particles in the light microscopic range.

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

However, it should be emphasized that even with this degree of destruction of fibrous structure, the animals still inhaled substantial quantities of apparently unchanged asbestos. It can be estimated that atmospheric concentration of the fibrous component alone in the chambers was 40-900 γ/m^3, and the fiber counts as reported above were 11-220 times higher than the current threshold limit value for asbestos as established by the American Conference of Governmental Industrial Hygienists (cf. Background, Reference 73). Moreover, a similar destruction of fibrous structure was not observed in the electron microscopic range; under the electron microscope it appeared that the majority of particles were fibrous and average length and diameter of dust samples collected in the chambers were as follows: amosite, 3-5 and 0.2-0.5; crocidolite, 3-6 and 0.4-0.5; and chrysotile, 6-15 and 0.2 μ.

We performed x-ray diffraction study of asbestos samples on a General Electric XRD-5 apparatus. Diffraction line positions agreed well with those reported in the literature (cf. Background, Reference 75). The loss of line intensity (measured as difference in peak heights on samples obtained before and after the dissemination procedure) was computed as per cent of the UICC reference value.

The results (Table 1) show about 70% loss of diffractivity for amosite, and about 35% for crocidolite and chrysotile. These values are uncorrected for the effect of particle size diminution and since these patterns were not yet compared with those of forsterite and pyroxene (heat decomposition products of asbestos), the figures are not immediately interpretable as per cent loss of crystallinity. Further studies in this direction are in progress.

TABLE 1

Sample	x-ray line (degrees)	UICC units	J-M crude units	J-M crude % of UICC	J-M disseminated units	J-M disseminated % of UICC	% loss of peak ht. during grinding	
[illegible]	10.7-10.9	78	78	100	30	38	62	av.
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	70%
[illegible]	[illegible]	39	41	105	10	26	[illegible]	
	32.3-32.8	8	10	125	3	38	70	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
	26.0-26.7	35	14	40	18	52	--	av.
Crocidolite	28.7-29.0	19	20	105	9	48	55	35%
	32.9-33.2	14	14	100	12	86	14	
	34.4-34.7	5	5	100	3	60	40	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
	14.0-14.4	50	50	100	37	74	32	av.
Chrysotile	18.7-19.8	10	10	100	6	60	40	35%
	24.3-24.7	32	40	125	16	50	60	
	60.2-60.7	6	6	100	5	83	17	

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002098

Another artefact introduced during dust preparation consisted of heavy metal contamination. The ballmilling procedure was accomplished with stainless steel balls of 1/2-2" diameter, supplied by the Atlas Ball Division of SKF Industries. The company gave the following analytical data for the balls: Cr 1.3-1.6%; C 0.95-1.1%; Si 0.2-0.35%; Mn 0.25-0.45%; Ni <0.35%; Cu <0.25%; Mo <0.08%; P <0.025%; S <0.025%. It is apparent that during the prolonged contact of the balls with the asbestos samples there was potential hazard of trace element adsorption.

We conducted analytical-chemical studies using atomic absorption spectrophotometry in order to compare raw and processed asbestos samples and to relate each to UICC standard reference samples. About 1 gm specimens of each dust were digested with the minimum amount of HF in Pt-crucibles and slowly evaporated to dryness. The ash was twice digested with conc. HNO_3 and then dissolved in 1 N HCl. The elements Co, Ni, Cr, Mn, and Fe were determined using the 2407, 2302, 3579, 4030, and 3470 Å resonance lines, respectively. Iron was also determined spectrophotometrically as hydroxylamine-HCl and o-phenanthroline complex. The results are summarized in Table 2.

It appears that with UICC samples, our results agreed well with those of Timbrell (cf. Background, References 75 and 76), and in most cases there was no great discrepancy between these values vs. those obtained on crude Johns-Manville samples. JM-3T chrysotile resembled UICC-B chrysotile, although its nickel concentration was appreciably lower.

Substantial contamination by chromium was introduced during grinding into amosite and crocidolite, and by nickel into crocidolite, the factors being 3X, 9X, [illegible] respectively. The other values appear to be within or close to experimental error limits.

TABLE 2

Specimen and method				Cr ppm	Mn ppm	Fe %	Co ppm	Ni ppm
		as reported	atomic abs.	32	13,000	15.1	11	34
[illegible]								
	Johns-Manville W-3		crude	40±3	14,000±400	24±1	10±1	52±2
			processed	120±5	14,000±400	25±1	12±1	58±2
	UICC A	as reported	atomic abs.	22	864	15.1	9	13
		by Timbrell	neutron act.	16	870	27	2	<100
Crocidolite		as analyzed in our lab		16±5	820±15	23±4	10±2	12±2
	Johns-Manville W-Blue		crude	13±1	1,030±50	33±6	6±1	12±1
			processed	120±7	1,320±70	33±6.5	7±1	58±2
	UICC A	as reported	atomic abs.	[illegible]	[illegible]	1.88	54	1445
		by Timbrell	neutron act.	1490	1430	1.70	55	1250
		as analyzed in our lab		1400±60	360±40	2.2±0.1	40±1	880±80
	UICC B	as reported	atomic abs.	316	443	1.14	45	795
Chrysotile		by Timbrell	neutron act.	490	510	2.60	46	990
		as analyzed in our lab		460±80	460±40	2.8±0.4	41±2	700±60
	Johns-Manville 3-T		crude	320±80	450±10	2.5±0.3	30±1	330±40
			processed	500±60	480±10	2.8±0.4	33±2	490±40

8000 2157

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002099

3. The Animal Colonies

207 Charles River CD rats, 96 Camm-Hartley guinea pigs, 60 Shankin Farms Dutch rabbits, 90 Stout Farms Webster mice, and 204 Hasenau Mongolian gerbils of both sexes were evenly divided among the amosite, crocidolite, and chrysotile chambers, respectively, with a smaller number (10-12 of each species) being retained as unexposed controls. The animals were 3-6 weeks old when shipped (during the summer of 1969); they entered the exposure chambers on December 22, 1969. Sample necropsies were obtained after 3 and 6 months, respectively, with the majority of animals allowed to survive the complete course of exposure which ended on December 21, 1971. Thereafter, animals were necropsied in batches throughout 1972. Altogether, out of a total animal count of 715, there were 417 animals sacrificed on schedule; 249 animals died in the course of the experiment through attrition; and 49 were lost to cannibalism or otherwise unaccounted for. Attritional losses were heaviest among rats during the fourth semester of exposure when 25-30 animals were lost for unexplained reasons. There were no indications of infectious epizootic and no antibiotic medication was given. It may be that the mortality was the consequence of fully developing pulmonary fibrosis, perhaps in combination with higher summertime temperatures. Mortality among the other animal species was normal, and there was no gross differential effect of exposure to any of the dusts on survival. The complete animal inventories are summarized in Table 3.

TABLE 3

Sample	Specimen Count		mice	gerbils	rats	rabbits	guinea pigs
Amosite	Inventory of live animals at	0 months	30	68	69	20	32
		6 months	23	64	62	14	20
		12 months	17	60	56	12	20
		18 months	0	[illegible]	46	10	13
		24 months	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	No. slides prepared		19	58	66	20	30
Crocidolite	Inventory of live animals at	0 months	30	68	69	20	32
		6 months	26	63	63	14	20
		12 months	18	58	57	12	20
		18 months	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		24 months	0	46	22	9	6
	No. slides prepared		22	49	66	20	31
Chrysotile	Inventory of live animals at	0 months	30	68	69	20	32
		6 months	26	64	62	16	22
		12 months	19	56	56	13	20
		18 months	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		24 months	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	No. slides prepared		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Control	Inventory of live animals at	0 months	10	12	12	12	12
		6 months	[illegible]	10	9	7	8
		12 months	6	10	7	6	8
		18 months	0	8	5	4	5
		24 months	0	8	3	3	4
	No. slides prepared		10	12	12	12	11
TOTAL NO. OF ANIMALS			100	216	219	72	108

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

4. Pathology Results

Histopathologic slides were obtained from the lungs and pleura of altogether 643 animals, with varying length of exposure. Hematoxylin-eosin, VanGieson's elastic, and $K_4Fe(CN)_6$ stains were performed on the sections. In general terms, the pathologic findings were the following (for a brief summary, see also Table 4):

a) Rats

Among control animals there was one bacterial pneumonia but no other significant pathology. Among exposed animals, histiocytic and giant cell response was universal and gradually increasing in the course of exposure. Fibrotic response was most severe in the crocidolite group. Fiber deposits were frequently seen but there were few readily apparent ferruginous bodies and almost no necrosis. A few cases of pneumonia and bronchitis were seen. In the crocidolite group, frequent pleural reaction with collagen accumulation occurred, as well as hemosideri accumulation in the lymphnodes. There was filling of some alveoli with histiocytes, giant cells, and cholesterol.

A proliferative response was especially frequent in the crocidolite group, where 8 animals (on the 567, 585, 691, 693, 727, 772, 779, and 785th day, respectively) had squamous metaplasia of the alveolar lining. In 2 crocidolite-exposed animals, epithelial papillomata were seen in the bronchi, which appeared histologically benign. There were also definite malignant neoplasms in all groups as follows:

(1) Amosite. Rat no. 1206-23, male, found dead after 539 days of exposure. The lung and pleura contained a large neoplasm of fibrosarcoma pattern with adhesions to the chest wall. Rat no. 1206-26, male, found dead after 564 day of exposure. The lung contained an [redacted] found dead on the 18th day after completion of exposure. The lung contained a bronchoalveolar carcinoma with papillary pattern, composed of tall columnar cells. A similar neoplasm was seen on the pleural surface.

(2) Crocidolite. Rat no. 1208-48, female, found dead on the 12th day after completion of exposure. The lungs contained a bronchogenic adenocarcinoma. Rat no. 1208-49, male, found dead on the 18th day after completion of exposure. The lungs contained a squamous cell carcinoma. Rat no. 1208-55, female, sacrificed 45 days after completion of exposure. The lungs contained a squamous cell carcinoma. Rat no. 1208-69, female, sacrificed 56 days after completion of ex- [redacted] posure. The lungs contained a papillary carcinoma. Rat no. 1207-61, male, sacrificed 43 days after completion of exposure. The lungs contained a well-differentiated squamous cell carcinoma.

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

U.S.GPO: 1970 - 370-[illegible]

8000 1159

HFM - 002101

b) Rabbits

In control animals there was no significant pathology. Amosite and crocidolite caused histiocytic and foreign body-responses, with the extent of reaction well correlated to the length of exposure. Light to intermediate fibrosis was observed in animals surviving the complete course of exposure. Inflammatory response was infrequent, and chrysotile caused remarkably fewer lesions. There were no malignancies.

c) Guinea Pigs

Control animals had no significant pathology. Among exposed animals, this species was remarkable for conspicuous abundance of ferruginous bodies, especially after crocidolite exposure. Foreign-body and giant-cell response, as well as gradually developing fibrosis, were common in all exposure groups. There were a few cases of adenomatosis but no malignant neoplasia.

d) Gerbils

Control animals had no significant pathology. Exposed animals showed foreign-body response, light to moderate fibrosis, and a conspicuously high incidence of focal and generalized alveolar proteinosis. Thus far no cases of malignant neoplasia were found, but examination of the material is not yet complete.

e) Mice

Examination of this slide material is in progress at the time of this writing and no data are available.

Lung Weight/Body Weight Ratios

In addition to microscopic slide examination, the degree of tissue impairment was also assayed as lung weight/body weight ratio. Previous experience has shown that pulmonary lesions of diverse etiology have an enhancing effect on lung weight and since sick animals typically become cachectic, lung weight/body weight ratios are particularly sharp indicators of respiratory health. Body weight is obtained at sacrifice. Lung weight is obtained after excision, in wet state, but blotted between sheets of filter paper. Values of good consistency can thus be obtained. In this study, the mean lung weight/body weight ratios (in mg/g, ± S.D.) of unexposed controls was found to be the following: gerbils, 6.7 ± 0.3; rabbits, 7.3 ± 3.2; rats, 8.5 ± 4.2; mice, 11.5 ± 1.5; and guinea pigs, 14.1 ± 2.5.

Table 4 shows that exposure to various kinds of asbestos has increased the mean lung weight/body weight ratio in each species in the following ascending order: chrysotile, amosite, and crocidolite. Severest effects were seen with gerbils (crocidolite-control difference t= 3.14, significant at 99.5% confidence level); and with rats (crocidolite-control difference t= 2.36, significant at 95% confidence level). With the other species, the differences were of low or borderline significance, and in each species amosite and chrysotile caused successively less effect than crocidolite. Tumor-bearing animals were excluded from this computation, so that the results are indicative of the degree and extent of asbestosis.

U.S.GPO: 1970 - 379-[illegible]

8000 1160

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002102

TABLE 4

GROUP		ASBESTOSIS: No. of specimens	ASBESTOSIS: LW/BW (mg/g ± SD)	ASBESTOSIS: microscopic appearance of slides	NEOPLASIA: No. animals surviving 18 months	NEOPLASIA: Incidence and type of malignancy
Gerbils	control	7	6.7±0.3	normal	8	
	chrysotile	40	6.4±1.6	histiocytes	50	
	crocidolite	31	15.3±7.0[a]	fibrosis alveolar proteinosis	49	none (incomplete)
	amosite	45	8.5±5.1	histiocytes fibrosis	51	
Rabbits	control	5	7.3±3.2	normal	4	
	chrysotile	11	5.8±0.9	near normal	11	
	crocidolite	10	8.0±1.0	histiocytes fibrosis	9	none
	amosite	9	5.3±1.3	histiocytes fibrosis	10	
Rats	control	6	8.5±4.2	normal	5	none
	chrysotile	34	8.8±5.1	histiocytes fibrosis	43	1 pap. ca. of lung 1 squ. ca. of lung 1 fibrous tumor(?) of mediastinum
	crocidolite	43	14±6.5[b]	many histiocytes; fibrosis	46	1 adenoca. of lung 3 squ. ca. of lung
	amosite	43	10.5±5.3	histiocytes	46	1 pap. ca. of lung 1 metast. osteosarc. of lung 1 fibrosarc. of lung and pleura 1 mesothelioma of pleura
Mice	chrysotile	1	10.6	not yet available	0	
	crocidolite	4	14.4±2.8		0	none
	amosite	5	12.2±3.1		0	
Guinea pigs	control	6	6.1±1.5	histiocytes	14	
	chrysotile	15	13.2±2.9	histiocytes slight fibrosis	14	
	crocidolite	13	8.5±5.5	histiocytes fibrosis	14	none
	amosite	15	14.4±3.6	histiocytes fibrosis	13	

[a] difference from control significant at 99.5% level ($t_{36df} = 3.14$).

[b] difference from control significant at 95% level ($t_{47df} = 2.36$).

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

8000 1161 PQ

HFM - 002103

6. Corollary Studies

A. Review of Chamber Dissemination Procedures.

It is apparent from Chapter 2 above that the chamber dissemination procedure applied in the inhalation experiment is in need of improvement. It appeared that the prolonged milling has caused considerable destruction of fibrous structure of the dusts as well as their enrichment in trace metals. Moreover, the large chamber size and the high atmospheric concentration required large quantities of asbestos which were not recoverable.

Pilot studies are underway to design a system in which these shortcomings are minimized. The UICC dust feeder of Timbrell et al. (cf. Background, Reference 77) is presently under test in conjunction with a 35" x 40" x 43" rectangular steel-and-glass inhalation chamber, which would allow simultaneous exposure of about two dozen rats and dust sediments would be recoverable. This is especially important in cases where the availability of dusts is limited, such as the chemically modified dusts (vide infra, Section D). However, such a system is suitable only for small animal colonies.

Improved utilization of our present walk-in chambers, suitable to the exposure of hundreds of animals, is also under exploration. A new dissemination unit is presently being tested which avoids hammer milling and utilizes a drum with 6" wire wheel, and a flat rasp-like surface ("Formafile" of the Stanley Tool Mfg. Co.) In this system, clumps of asbestos mutually erode each other and much of the fiber destruction and trace metal contamination which has plagued previous experiments would be eliminated.

Analytical-chemical, x-ray-crystallographic, and microscopic particle size studies on these newly created asbestos dust clouds are presently underway and at the time of this writing no results are as yet available.

B. Fiber Length and Severity of Fibrosis.

A brief investigation resulting in a master's thesis (cf. Appendix [illegible]) was completed on this topic in conjunction with the main project. Crocidolite (JM S-blue, cf. Chapter 2 above) was subjected to graded ballmilling of specified duration and the resultant samples intratracheally injected into groups of rats. Untreated controls, and india ink-injected animals (for distribution studies after intratracheal injection) were also included. The results were evaluated during necropsy at 3, 6, and 9 months after the injection, according to the following criteria: (1) degree of alveolar wall thickening; (2) hemosiderin content; (3) collagen content; (4) average number of leucocytes per fibrotic nodule; and (5) average number of alveoli involved in a fibrotic nodule. The results showed that ballmilling, although it appeared to destroy the fibrous structure of asbestos in the light microscopic range, it did not destroy its fibrogenicity. Asbestos particles below 5 μ were at least as fibrogenic as larger asbestos particles. The degree of fibrosis was essentially steady rather than progressive from 3 to 9 months after a single intratracheal injection. Certain parameters such as dust count, hemosiderin concentration, and leucocyte population tended to diminish with time.

☆U.S.GPO: 1970 – 379-[illegible]

8000 1162

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002104

C. Ferruginous Body Counts and Severity of Fibrosis.

Previous investigations (cf. Background, References 7, 16, 17, 18, 25, 74) have suggested that ferruginous body formation may be a protective mechanism of pulmonary tissue against asbestos fibers. If that is the case, ferruginous body counts and degree of fibrosis may show inverse correlation in various individuals. Ferruginous body abundance seems to be species-dependent and it was noted that rats, with pronounced fibrotic response to asbestos and consistent incidence of neoplasia, showed few ferruginous bodies whereas guinea pigs, with lesser severity of asbestotic fibrosis and no cases of neoplasia, showed a great abundance of them.

A ferruginous body count experiment is currently in progress on several animal species at various stages of asbestotic fibrosis. 1 gm aliquots of pulmonary tissue are being digested in NaOCl solution, the ferruginous bodies collected on millipore filter, and counted under the microscope (69). The counts will be correlated to the degree of pathologic changes in the lung. These studies are at present unfinished.

D. Trace Metal Removal From Asbestos Fibers.

Attempts were made to eliminate potentially carcinogenic trace elements from asbestos. Fiber specimens were incubated with chelating agents such as 1% EDTA, or with hydrochloric acid solutions in the concentration range of 0.1-5.0 N. Incubations were continued for 3 days and the residue submitted to x-ray crystallography. Trace metals recovered in the leaching solutions are tabulated in Table 5. These figures are as yet uncorrected for the solubility of asbestos fibers in the incubation media which, especially in the case of chrysotile with strong acid, is quite high. The experiments are continued, involving higher concentration of chelating agents, and correction of metal removal figures for fiber solubility. The 1 day 1 N HCl residue (Ni concentration [illegible] 15-47%) is currently being tested for carcinogenicity, in comparison to crude fibers, by intraperitoneal injection into groups of rats (vide infra, Section E).

E. Assessment of Carcinogenicity of Treated Asbestos Fibers.

The intraperitoneal injection method which in previous experiments was shown to yield a very high yield of mesotheliomas with asbestos, (cf. Background, References 55 and 56) was used as a model system for the evaluation of the carcinogenic capacity of asbestos specimens where the available quantity was limited. Twenty [illegible] of both sexes, about [illegible] months old, were used per sample. Necropsies are scheduled at 18 months after injection at which time the tumors will be well developed according to previous experience. Pre-dissemination samples, post-dissemination samples, and UICC reference samples of amosite, crocidolite, and chrysotile are involved in this experiment. Controls received similarly injected glass wool. Also tested were specimens obtained in the trace metal-removal experiments described in Section D above. These experiments are currently in progress and no results are as yet available.

U.S.GPO: 1970 - 379-[illegible]

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

8000 2163

HFM - 002105

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

TABLE 5

Fiber type	Agent	Leaching time (days)	Av. int. x-ray lines, % of crude	% metal content removed				
				Cr	Mn	Fe	Co	Ni
Amosite	1% EDTA	1	95	4.5	3.0	0.9	7.0	10
		2	103	7.5	3.5	0.9	9.0	10
		3	95	7.5	4.3	1.3	9.0	10
	0.1 N HCl	1	100	7.5	4.4	2.0	0	0
		2	109	7.5	4.9	2.3	00	4.0
		3	105	7.6	5.0	2.3	3.0	4.0
	1 N HCl	1	117	15	5.7	4.0	0	12
		2	123	30	6.0	4.7	7.0	12
		3	107	30	6.2	4.9	9.0	12
	5 N HCl	1	112	30	6.3	6.7	12	15
		2	125	40	7.7	8.4	12	15
		3	38	40	7.7	8.4	12	17
Crocidolite	1% EDTA	1	84	22	2.9	2.0	10	50
		2	84	23	2.9	2.2	10	55
		3	80	23	2.9	3.3	10	60
	0.1 N HCl	1	82	23	46	7.8	0	25
		2	77	23	48	3.0	0	25
		3	78	24	50	3.2	0	25
	1 N HCl	1	82	20	54	4.6	15	41
		2	71	23	55	5.6	15	50
		3	80	25	57	5.7	5	50
	5 N HCl	1	73	31	61	12	10	35
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		3	[illegible]	[illegible]	[illegible]	12	17	53
Chrysotile	1% EDTA	1	84	13	40	10	10	8.0
		2	103	13	40	10	10	8.0
		3	102	13	40	10	10	8.0
	0.1 N HCl	1	106	1.8	45	4.0	35	70
		2	[illegible]	1.8	45	4.0	35	100
		3	87	2.0	46	4.0	35	100
	1 N HCl	1	50	47	86	42	75	100
		2	55	51	100	50	88	100
		3	48	56	100	55	90	100

[redacted]

U.S.GPO: 1970 - 379-[illegible]

8000 1164

HFM - 002106

D. Publications

1. Reeves, A. L., Puro, H. E., Smith, R. G., and Vorwald, A. J.: Experimental Asbestos Carcinogenesis. Environ. Research 4, 496-511 (1971).

2. Davis, H. V. and Reeves, A. L.: Collagen Biosynthesis in Rat Lungs During Exposure to Asbestos. Am. Indust. Hyg. Assoc. J. 32, 599-602 (1971).

3. Ford, J. E.: The Effect of Pulmonary Macrophage Suppression in Developing Asbestosis. Dissertation, Wayne State University, Detroit, 1971.

4. Olajos, E. J.: Asbestosis and Fiber Length. Thesis, Wayne State University, Detroit, 1972.

5. Reeves, A. L., Puro, H. E., Smith, R. G., and Vorwald, A. J.: Fibrogenicity and Carcinogenicity of Amosite, Crocidolite, and Chrysotile in Animal Experiment. Am. Indust. Hyg. Assoc. J. 33(2), A 79 (1972).

6. Reeves, A. L., Puro, H. E., and Reeves, E. H.: Fibrogenesis and Carcinogenesis From Various Forms of Asbestos. Proc. 17th Internat. Congr. Occup. Health inpress.

7. Roy-Chowdhury, A. K., Mooney, T. F., and Reeves, A. L.: Trace Metals in Asbestos Carcinogenesis. Arch. Environ. Health in press.

E. Staffing

Principal Investigators

Arthur J. Vorwald, M. D., Ph. D., Professor and Chairman (6/1/66-11/15/67)
Ralph G. Smith, Ph. D., Professor and Acting Chairman (11/15/67-7/31/70)
Andrew L. Reeves, Ph. D., Professor (8/1/70-present)

Pathologist

Henry E. Puro, M. D., Associate Professor (1/15/68-present)

Engineer

Duane VanAcker, Instrumentation Technician (6/1/66-present)

Chemists

Thomas F. Mooney, Jr., Ph. D., Assistant Professor (8/1/70-7/31/72)
Anil K. Roy-Chowdhury, Ph. D., Research Associate (12/1/71-present)

Participating Graduate Students

Harvey V. Davis, Ph. D. (6/1/66-5/31/69)
Jon E. Ford, Ph. D. (6/1/69-5/31/71)
James P. Burger, M. S. (6/1/69-5/31/70)
Eugene J. Olajos, M. S. (9/1/71-8/31/72)
Steven Wainess, M. S. candidate (9/1/71-present)

☆U.S.GPO: 1970 – 379-585

8000 1165

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002107

B. Specific Aims

Experiment 1. To continue with trace metal removal experiments from asbestos fibers and test the resulting dusts for biological activity. Desorption, chelation, and ionic exchange will be used to prepare additional specimens of amosite, crocidolite, and chrysotile. Particle size by microscopy and crystallographic structure by x-ray diffraction will be determined. Biologic assay will be by intraperitoneal injection into rats.

Experiment 2. To perform solvent extraction experiments on asbestos fibers and test the resulting dusts for biological activity. This study is indicated in view of the complete contradiction between the results of Harington et al. and Pott et al. on one hand and Roe and Rylev on the other hand (cf. Background, References 35, 54, 59, 61). Dusts artifically enriched in 3, 4-benzpyrene will also be included, and tested by intraperitoneal injection into rats.

Experiment 3. To study the effects of heat on the crystal structure and biological activity of asbestos fibers and asbestos-containing commercial products. Specimens of crude asbestos, heat-treated asbestos, brake shoe scrapings, and brake lining dust as collected in brake drums will be subjected to comparative x-ray diffraction studies and to biological assays by the intraperitoneal injection method in rats.

Experiment 4. To assess the importance of mechanical irritation in asbestos-induced pleural neoplasia. The pleura and peritoneum are believed to be specifically vulnerable to the effects of asbestos because of the mechanical irritation to the surfaces during respiratory movements. Such mechanical effects vs. the physicochemical factors discussed above are postulated in competing hypotheses of asbestos carcinogenesis. The mechanical irritation [redacted] by administering bilateral intrapleural injections of asbestos dust to miniature pigs and then laying one side of the chest to rest by induced unilateral pneumothorax. If mechanical irritation is an important factor in asbestos carcinogenesis, there should be a difference in the degree of malignant response [redacted] sides.

Experiment 5. Inhalation exposure of limited animal colonies in small chambers to dusts which in the preliminary trials (Experiments 1-3 above) appear to have lost some or all of their carcinogenic activity. This confirmation is necessary to establish that certain modified asbestos dusts (metal-stripped, solvent-extracted, or heat treated) are no longer hazardous upon inhalation. For small quantities of dusts, the "Konimiser" (cf. Background, Reference 40) is a suitable dissemination system. Rats are suitable experimental animals, and exposure will last 2 years.

Experiment 6. To test, by inhalation exposure, automobile brake emissions under simulated use conditions. A dissemination system involving a dynamometer will be built for the purpose of dispersing into inhalation chambers the actual dust created during brake operation. The brakes will be operated intermittently, in stop-and-go fashion, 4 hrs. daily. A colony of rats will be exposed to the inhalation of these dusts for a period of 2 years.

8000 f166

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002108

Experiment 7. To repeat, with certain modifications, the large inhalation experiment described in the attached Progress Report, and to investigate by biochemical means the differential susceptibility of various animal species to asbestos cancers. An improved dissemination system which will minimize fiber destruction and trace metal contamination has been developed and is now under testing (cf. Progress Report, p. 30). Large colonies of rats and guinea pigs (the two species found to be most and least vulnerable to asbestos cancers, respectively) will be exposed to the inhalation of chrysotile, crocidolite, or amosite, allowing periodic serial sacrifices during exposure. Pulmonary macrophages and pulmonary tissue homogenates will be studied by starch gel electrophoresis and proline hydroxylase assay (cf. Biographical Sketches, p. 6-7, References 11, 12, 13, 16, 19, and 23), in order to pinpoint any differences which might be responsible for the different response of these animals to asbestos inhalation.

C. Methods of Procedure

1. Animals. The Charles River CD rat was found to be most suitable for asbestos carcinogenesis work, both by inhalation and implantation. These animals will be used in Experiment 1-3, 5, and 6. Experiments 4 will be performed on miniature pigs ("Pigmy-Pigs", Hormel Inst., Univ. Minnesota). In Experiment 7, rats and Camm-Hartley guinea pigs will be used for biological comparison. This laboratory has had adequate experience with keeping miniature pigs and guinea pigs. Adequate quarantine periods will be observed. Preventive antibiotic medication may be given as needed in case of a threatening epizootic.

2. Intraperitoneal injections (Experiments 1-3). This simple technique, [redacted] mortar, suspended in isotonic saline at a concentration of 50 mg/ml, and sterilized, has been used routinely in this laboratory (cf. Background, Reference 55). One ml doses are given to rats; no reinjection is required. Evidence of reaction is obtained after 7-13 months.

3. Unilateral pneumothorax (Experiment 5). This technique was tried on various animals in a pilot study. It was possible to induce unilateral pneumothorax through slow and careful intrapleural injection of air, but in rodents (rats, rabbits and guinea pigs) the mediastinum is very thin and ruptured easily, leading to frequent expiration of an animal. On the other hand, miniature pigs could sustain a pneumothorax with ease for long periods. These animals are expensive, but only a few are required since each animal can serve as its own control (with pleural [redacted] receive injection of air is planned with subsequent x-ray check on the success of the operation, so that one side of the chest is laid to rest throughout the required induction period.

4. Large inhalation chambers (Experiment 7). Reorganization of our present exposure facility is now in progress (cf. Progress Report, p. 30). We are aiming at a dissemination technique with less fiber destruction and less trace metal contamination. Concurrently, the atmospheric concentration will be reduced to 5 mg/ml. Aerosols of amosite, crocidolite, and chrysotile will be generated.

8000 1167 PQ

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002109

5. Small inhalation chambers and brake line dust dissemination (Experiment 6). This Department has four 35" x 40" x 43" rectangular steel-and-glass inhalation chambers, two of which will be fitted with an aerosol generating system involving commercial brake shoes and simulated brake operation. A dynamometer will be built, fitted with brake drums, and assembled in such way that actual brake linings are being pulverized during operation. The operation will be "stop-and-go" rather than "dragging" in order to approach the actual conditions of emission, and the created dust will be blown into the chambers at a low air flow rate in order to achieve maximum concentration. The details of the apparatus will have to be worked out empirically since no such instrumentation exists today to our knowledge anywhere; but we have seen dynamometric brake-test setups built for different purposes at Bendix Corp. Research Laboratories, Southfield, Michigan, and at Ford Motor Co., Engineering Laboratories, Dearborn, Michigan (cf. Collaborative Arrangements). It is our hope to achieve an atmospheric brake emission concentration of about 5 mg/ml in the chambers. Renewal of the brake shoes twice a month and of the brake drums twice a year is envisioned.

6. Preparation and dissemination of modified dusts. Current experiments on trace metal removal with dilute acid and chelating agents will be continued. Also explored will be ionic exchange procedures in order to replace carcinogenic metals (Ni, Cr, Co? Mn?) with noncarcinogenic metals of suitable ion radii (Al, Sc, Ca? Mg?). Tolerances of ion radius difference for successful exchange are unknown at present and must be established by experience. Solvent-washed specimens and heat-treated specimens (Experiments 2 and 3) will be prepared, and brake lining dust collected in brake repair shops will be included. Testing of these specimens will be first by the intraperitoneal injection method (Experiments 1-3). Where indicated, limited inhalation exposures (Experiment 5) will be set up in the two remaining rectangular chambers, in combination with Hounam's "Konimiser" or Timbrell's UICC dispenser (cf. Background, References 40 and 77). The latter is currently being tested in our laboratory (cf. Progress Report, p. 30).

7. Necropsies. Animals will be sacrificed for study at appropriate end points (about 12-18 months after implantation, or about 6 months after a 2-year inhalation exposure) except in Experiment 7 where bimonthly serial sacrifices throughout the experiment are required. Necropsies will be accomplished in Surital anesthesia and the animals will be dissected. Body weights at sacrifice and lung weights after excision and blotting will be obtained.

8. Histopathology. Pulmonary, pleural, and peritoneal specimens are fixed in formalin and stained with hematoxylin-eosin and/or $K_4Fe(CN)_6$-basic fuchsin [illegible] histopathology is studied and evaluated on a scale 0-5 in the following categories: Inflammatory cells; Histiocytes; Giant cells; Necrosis; Fibrosis; Fibrous proliferation; Mesothelial proliferation; Epithelial proliferation; Metaplasia; and Neoplasia. Tumor types are described as usual.

9. Tumor transplants. Malignant tumors are characterized by transplantability and the degree of malignancy can be expressed as the rate of growth after transplant. Thus far, our attempts to transplant asbestos-induced tumors into host animals were not successful. This was apparently due to failure to recognize incipient gross tumors at autopsy. We shall continue our efforts in that regard.

8000 1168

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002110

10. Biochemical studies. Experiment 7 will involve serial sacrifices of rats and guinea pigs, throughout asbestos exposure in order to discover why one species does develop cancer while the other does not. Pulmonary tissue homogenates (cf. Biographical Sketches, p. 6 , Reference 11) and harvested alveolar macrophages (cf. Background, Reference 49) will be obtained and subjected to starch gel electrophoresis according to Reeves et al. (l. c.) and to proline hydroxylase assay according to Davis and Reeves (cf. Background, Reference 15). On starch gels, staining techniques for protein, seromucoid, acid and alkaline phosphatase, lactate and malate dehydrogenase, and esterase are current routine in our laboratory. Further stains may be developed from histological staining processes if indicated. Proline hydroxylase activity is an index of the rate of collagen biosynthesis which in turn accompanies the fibrotic transformation. With this technique, it may be possible to test the assumption that malignant transformation is dependent on previous fibrosis (cf. Background, Reference 9), and differences between rat and guinea pig zymograms during exposure to asbestos may indicate the reason(s) for the divergent pathologic response.

11. Chemical and crystallographic studies. Emission spectrography, atomic absorption spectrophotometry, and x-ray diffraction analysis (cf. Facilities Available) will be used for the study of modified asbestos and brake emission specimens. Data of the kind listed in the Progress Report, Tables 1, 2, and 5 will be obtained.

12. Light and electron microscopy. In addition to the use of these instruments in histopathology, complete aerosol characterization will be performed on air samples from the chambers in Experiments 5, 6, and 7. The samples will be collected by the membrane filter method which gives a representative size frequency distribution.

13. Data treatment. All results, where applicable, will be examined statistically. Number of controls and test animals in each experiment will be comparable, and differential incidence of neoplasia or other unusual pathology will be examined by the chi-square test. Lung weight-body weight ratios and other quantifiable measurements will be examined by the t-test. Correlations will be examined by [redacted] analysis.

14. Timetable. Experiment 1 is currently in progress and will be continued without interruption if renewal is granted. The first batch of test rats will come to necropsy by July 1973; further batches will be injected as additional samples from Experiments 1, 2, and 3 become available. Experiment 4 (on mini-pigs) will be commenced in the fall of 1973 and the animals will be ready for inspection in 1976. Experiment 7 (restart of large inhalation chambers) is also scheduled for the fall [redacted] preparatory engineering work is now at an advanced stage of progress. Exposures will be completed by fall of 1975, with final necropsies and specimen processing carried over into 1976. Experiment 5 (small-scale exposures to modified dusts) will not be started until 1974 since preliminary indications are required as to which dusts from Experiments 1, 2, and 3 appear to have diminished carcinogenic potencies. With a 2-year exposure period and one additional year for necropsies, tissue processing and study, this experiment will last until 1977. Experiment 6 (brake emission study) requires a one-year tool-up and exposures will be started in 1974; concluded in 1976; and specimen study completed by 1977. Accordingly, we seek support for all these studies for a period of 4 years.

8000 1169

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002111

D. Significance

Biological comparisons of various mineralogical forms of asbestos in various animal species, involving two large inhalation experiments (cf. Appendixes 1, 2, 3, and the Progress Report) and intratracheal, intrapleural, and intraperitoneal experiments (cf. Appendixes 4, 5, and 6) now stand concluded. In this application, we are proposing new ventures in order to answer new questions. Elimination of the carcinogenic hazard originating from asbestos requires understanding of the carcinogenic principle, and in an interlocking and mutually supporting set of experiments we wish to test the theory of mechanical irritation (Exp. 4), the theory of trace metals (Exp. 1) and the theory of organic contaminants (Exp. 2). We also propose to study the biochemical indices of progressing response to inhaled asbestos, and compare cancer-prone and non-cancer-prone species in this respect (Exp. 7).

In addition, we also propose to find out what happens to the carcinogenic principle in asbestos during heat treatment, both under laboratory conditions (Exp. 3) and under simulated use conditions of an important asbestos-containing commercial product (Exp. 6). Specimens with apparently diminished carcinogenic potential will be re-tested in inhalation experiment (Exp. 5). Some of these experiments require long waiting periods between treatment and observation, while others necessitate near-continuous activity at certain times. Simultaneous performance of these experiments will provide optimum manpower utilization.

The public health significance of these studies is obvious. We hope to resolve the question of urban carcinogenic hazard from vehicle brake emissions and to indicate what, if any, modifying treatment will reduce the carcinogenic potential of asbestos fibers. We shall also attempt to identify the reason why one species develops cancer from asbestos while another does not. These questions have great importance in the protection of human populations against the carcinogenic effects of asbestos inhalation.

E. Facilities Available

All experimental work will be performed on the premises of the Department of Occupational and Environmental Health, Wayne State University, which is well equipped to conduct this type of investigation. Three large exposure rooms (walk-in chambers) are available, [illegible] fitted with a new dissemination system which will reduce fiber destruction and trace metal contamination. Four racks, each holding 48 rats or 36 guinea pigs, is the maximum capacity of [illegible] chambers as described (cf. Progress Report, p. 30) are available, located in suitable space to install the brake emission dynamometer (cf. Methods of Procedure, Section 5). Holding rooms for intrapleurally and intraperitoneally treated animals, including pens for mini-pigs, are available. Fully equipped animal surgery and necropsy room is available.

The chemistry laboratory has a Beckman DK-2 spectrophotometer, Perkin-Elmer atomic absorption spectrophotometer, Bausch & Lomb emission spectrograph, and G. E. XRD-5 x-ray diffraction apparatus. The biochemistry laboratory has refrigerated work area, a Lourdes LR high-speed refrigerated centrifuge, and full gel electrophoresis equipment. The histopathology laboratory is completely

8000 1170

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

HFM - 002112

equipped with various ovens and microtomes, a Leitz Ortholux microscope and a Zeiss photoscope, both with apochromatic optics. The Department also possesses an RCA Model EMU-3E electron microscope.

F. Collaborative Arrangements

Commercial asbestos samples are being donated to us by the Johns-Manville Corporation, courtesy Dr. S. Speil. Brake shoes for the brake emission inhalation experiment will be donated to us by the Bendix Corporation, courtesy Dr. W. M. Spurgeon. Engineering consultation is being offered to us in the building of the dynamometer and associated chamber exposure facility, by R. L. Gealer and A. Anderson of the Ford Motor Company.

Dr. H. E. Puro, Department of Pathology, is being assigned to serve with the Department of Occupational and Environmental Health, 25% of time, for the purpose of collaboration on this project, as pathologist. Veterinary consultation is available from the Department of Animal Resources of this School, Dr. A. G. Edward, Chairman. Microscopic, analytical-chemical, biochemical, and engineering work will be performed by full-time departmental staff as assigned to this project.

G. Appendix

1. Published paper, "Experimental Asbestos Carcinogenesis" by A. L. Reeves, H. E. Puro, R. G. Smith, and A. J. Vorwald. Environ. Research 4, 496-511 (1971).

2. Published paper, "Collagen Biosynthesis in Rat Lungs During Exposure to Asbestos" by H. V. Davis and A. L. Reeves. Am. Indust. Hyg. Assoc. J. 32, 599-602 (1971).

3. Doctoral dissertation, "The Effect of Pulmonary Macrophage Suppression in Developing Asbestosis" by J. E. Ford (R. G. Smith, Advisor). Wayne State University, Detroit, 1971.

4. Master's thesis, "Asbestosis and Fiber Length" by E. J. Olajos (A. L. Reeves, Advisor). Wayne State University, Detroit, 1972.

5. Manuscript, "Fibrogenesis and Carcinogenicity from Various Forms of Asbestos" by A. L. Reeves, H. E. Puro, and E. H. Reeves. Proc. XVIII. Internat. Congr. Occup. Health In press.

6. Manuscript, "Trace Metals in Asbestos Carcinogenesis" by A. K. Roy-Chowdhury, T. F. Mooney, Jr., and A. L. Reeves. Arch. Environ. Health In press.

DO NOT TYPE IN THIS SPACE-BINDING MARGIN

8000 1171

HFM - 002113