



DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20205

July 20, 1981

Mr. William R. Bibb
Director, Research Division
Department of Energy
Oak Ridge, TN 37830

Dear Mr. Bibb:

The application entitled "Neoplasia in human respiratory tissues - in vitro model" submitted on behalf of Dr. Margaret Terzaghi-Howe was received in the Division of Research Grants on July 17, 1981. It has been noted that the face page of this application does not have the signature of the person authorized to sign for the sponsoring institution. We regret to inform you that we are unable to accept applications without appropriate signatures. For your convenience the face page of the application is enclosed. We would appreciate having it returned to this office as soon as possible since official receipt of the application for possible consideration in this round of meetings is determined by the date of receipt of the signed proposal.

Sincerely yours,

Irene G. Lyddane
Grants Clerk
Referral Branch
Division of Research Grants

Enclosure

cc: Dr. Margaret Terzaghi-Howe

*Signed Original
Sent to NIH
7-23-81.*

*Rabin
40-1175-11*

Q 3542

1078951

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE GRANT APPLICATION JUL 17 1981 FOLLOW INSTRUCTIONS CAREFULLY	LEAVE BLANK		
	TYPE	ACTIVITY	NUMBER
	REVIEW GROUP		FORMERLY
	COUNCIL BOARD (Month, year)		DATE RECEIVED

1. TITLE OF APPLICATION (Do not exceed 56 typewriter spaces)
 Neoplasia in human respiratory tissues - in vitro model

2. RESPONSE TO SPECIFIC PROGRAM ANNOUNCEMENT NO YES (If "YES," state RFA number and/or announcement title)

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR

3a. NAME (Last, first, middle) 3b. SOCIAL SECURITY NUMBER
 Terzaghi-Howe, Margaret [REDACTED]

3c. MAILING ADDRESS (Street, city, state, zip code) 3d. POSITION TITLE
 Oak Ridge National Laboratory Staff Scientist
 Biology Division
 P.O. Box Y
 Oak Ridge, Tennessee 37830

3e. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT
 Biology Division

3f. TELEPHONE (Area code, number and extension) 3g. MAJOR SUBDIVISION
 (615)574-3971 Cancer and Toxicology Section

4. HUMAN SUBJECTS, DERIVED MATERIALS OR DATA INVOLVED 5. RECOMBINANT DNA RESEARCH SUBJECT TO NIH GUIDELINES
 NO YES (If "YES," form HHS 506 required) NO YES

6. DATES OF ENTIRE PROPOSED PROJECT PERIOD (This application)
 From: Dec. 1981 Through: Dec. 1984

7. TOTAL DIRECT COSTS REQUESTED FOR PROJECT PERIOD (from page 5) 8. DIRECT COSTS REQUESTED FOR FIRST 12-MONTH BUDGET PERIOD (from page 4)
 \$ 390,659 \$ 118,029

9. PERFORMANCE SITES (Organizations and addresses)
 Biology Division
 Oak Ridge National Laboratory
 P.O. Box Y
 Oak Ridge, TN 37830

10. INVENTIONS (Competing continuation application only)
 Were any inventions conceived or reduced to practice during the course of the project?
 NO YES - Previously reported
 YES - Not previously reported

11. APPLICANT ORGANIZATION (Name, address, and congressional district)
 Oak Ridge National Laboratory
 P.O. Box Y
 Oak Ridge, TN 37830
 Third Congressional District

12. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR INSTITUTIONAL GRANT (See instructions)
 Code 6 0 Description:

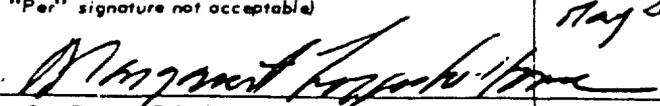
13. ENTITY IDENTIFICATION NUMBER
 60 - Department of Energy

14. TYPE OF ORGANIZATION (See instructions)
 Private Nonprofit
 Public (Specify Federal, State, Local):

15. OFFICIAL IN BUSINESS OFFICE TO BE NOTIFIED IF AN AWARD IS MADE (Name, title, address and telephone number.)
 William R. Bibb
 Director, Research Division
 Department of Energy
 Oak Ridge, TN 37830
 FTS 626-0727, (615) 576-0727

16. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Name, title, address and telephone number)
 William R. Bibb
 Director, Research Division
 Department of Energy
 Oak Ridge, TN 37830
 FTS 626-0727, (615) 576-0727

17. PRINCIPAL INVESTIGATOR, PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PERSON NAMED IN 3a (In ink. "Per" signature not acceptable)


DATE
 May 20/81

18. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense. (U.S. Code, Title 18, Section 1001.)

SIGNATURE OF PERSON NAMED IN 16 (In ink. "Per" signature not acceptable)
 William R. Bibb

DATE
 7-23-81

1078952

ABSTRACT OF RESEARCH PLAN

NAME AND ADDRESS OF APPLICANT ORGANIZATION (Same as Item 11, page 1)

Oak Ridge National Laboratory, Biology Division,
P.O. Box Y, Oak Ridge, TN 37830

TITLE OF APPLICATION (Same as Item 1, page 1)

Neoplasia in human respiratory tissues - in vitro

Name, Title and Department of all professional personnel engaged on project, beginning with Principal Investigator/Program Director

M. Terzaghi Staff Scientist
 Biology Division
 Cancer and Toxicology

A. J. P. Klein-Szanto Medical Staff Scientist
 Biology Division
 Cancer and Toxicology

ABSTRACT OF RESEARCH PLAN: Concisely describe the application's specific aims, methodology and long-term objectives, making reference to the scientific disciplines involved and the health-relatedness of the project. The abstract should be self-contained so that it can serve as a succinct and accurate description of the application when separated from it. DO NOT EXCEED THE SPACE PROVIDED.

In the proposed experiments we will evaluate the cytotoxicity and carcinogenicity of asbestos alone and DMBA adsorbed to asbestos in rat tracheal epithelium and human respiratory epithelium. Human respiratory epithelium cultured in vivo on devitalized rat tracheal stroma implanted into the nude mouse and tracheal epithelium will be exposed in vitro and maintained in organ culture up to 4 wks. At various times after exposure tissues will be fixed and presence of preneoplastic lesions as well as changes in the fraction of ³HTdr labeled cells determined. At the same time intervals cells will be harvested from exposed explants and tested for the capacity to grow in soft agarose (EF assay) or yield tumors when implanted into nude mice. The carcinogenicity will be evaluated, if possible at equal dose levels and equitoxic dose levels in rat tissues and in the respiratory tissues of at least four different humans. Of interest are both interspecies and intraspecies (human) differences in cytotoxicity and carcinogenicity of asbestos and DMBA. Original contributions made by these experiments will be the (1) establishment of experimental procedures which can be applied in future in vitro studies of neoplastic development in human respiratory epithelium following exposure to other potentially carcinogenic agents and (2) direct evaluation of the effects of asbestos and DMBA on normal "cultured" human respiratory.

LABORATORY ANIMALS INVOLVED. Identify by common names. If none, state "none"

Fischer 344 rats and CC3-10 nude mice

JUL 8 1981

Division of Research Grants
National Institutes of Health
Westwood Building, Room 240
5833 Westbard Avenue
Bethesda, Maryland 20205

Gentlemen:

ORNL GRANT PROPOSAL, "NEOPLASIA IN HUMAN RESPIRATORY TISSUES - IN VITRO MODEL" (INTERAGENCY AGREEMENT DOE NO. 40-1176-81)

Enclosed for your consideration are six copies of the subject research proposal, prepared by the DOE's Oak Ridge National Laboratory (ORNL). Also enclosed are an executed Grant Certification and an executed Cost Sharing Agreement in the format of HEW Form 490.

The proposed work involves studying the cytotoxicity and carcinogenicity of asbestos and asbestos with adsorbed DMBBA in rat and human respiratory epithelium in vitro. The proposal requests a three-year period of performance beginning December 1, 1981. The initial twelve-month funding will require \$118,029 for direct costs.

Your favorable consideration of the proposal should be reflected in a funding authorization to this office. Upon its receipt, we will authorize ORNL to proceed with the research project. Monthly billings will be accomplished by Standard Form 1001 to reimburse the DOE appropriation for actual cost incurred.

B 2474

40-1176-81

COST SHARING AGREEMENT

Awarding Office:

Application or Award Number:

Applicant:

U. S. Department of Energy
Oak Ridge Operations Office
Research Division
Post Office Box E
Oak Ridge, Tennessee 37830

Proposal Project Period:

From December 1, 1981, to November 30, 1984

ORNL RESEARCH GRANT PROPOSAL, "NEOPLASIA IN HUMAN RESPIRATORY TISSUES - IN VITRO MODEL" (INTERAGENCY AGREEMENT DOE NO. 40-1176-81)

In order to satisfy HEW cost sharing requirements, the U. S. Department of Energy agrees to bear 17.18 percent of the total cost of the above project.

Total project cost is the sum of the total allowable direct and indirect cost incurred by the recipient and sub-recipients of cost-type contractors, plus the value of any third party in-kind contributions which benefit the project. These amounts will be calculated according to the rules in the HEW grant administration regulations at 45 CFR, Part 74.

If the project period consists of more than one budget period, the agreed-upon rate will apply to the project period as a whole, including any non-competitive extensions but not necessarily to each individual budget period.

If HEW stops funding this project prior to the close of the originally agreed-upon project period, the rate will apply to the actual period of support.

If the project period is extended competitively, another cost sharing agreement will be negotiated.


William R. Bibb, Director
Research Division

7-8-81

Date

1078956

Certification Required by PHS: 1-515-40B

for

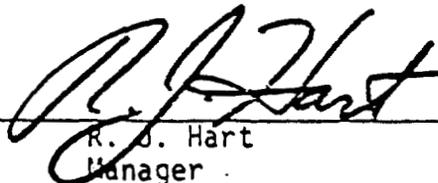
NEOPLASIA IN HUMAN RESPIRATORY TISSUES-- IN VITRO MODEL

To Be Performed By

The Department of Energy's Oak Ridge National Laboratory

I certify that:

1. The Department of Energy has the statutory authority to carry out the research study set forth in the proposal for the research project with the above title.
2. The Department of Energy will be legally, financially and administratively responsible for the conduct of the research project with the above title if a research grant is awarded to Oak Ridge Operations of the Department of Energy for this purpose.
3. Dr. William R. Bibb, Director, Research Division, Oak Ridge Operations, is delegated by me to administer the grant for the Department of Energy.



R. J. Hart
Manager
Oak Ridge Operations
Department of Energy
JUL 8 1981

OAK RIDGE NATIONAL LABORATORY

OPERATED BY
UNION CARBIDE CORPORATION
NUCLEAR DIVISION



POST OFFICE BOX X
OAK RIDGE, TENNESSEE 37830

OFFICE OF THE DIRECTOR

June 8, 1981

*Approved by
Maddox
7/8/81*

Department of Energy
Oak Ridge Operations
Attention: Mr. J. A. Lenhard, Assistant Manager
for Energy Research and Development
Post Office Box E
Oak Ridge, Tennessee 37830

Gentlemen:

Research Grant Proposal to the NIH, "Neoplasia In Human Respiratory
Tissues - In Vitro Model"
Interagency Agreement No. (40-1176-81)

Enclosed for your review and approval is a research grant proposal being submitted to the National Institutes of Health. The proposal requests a three-year period of performance beginning December 1, 1981, and a total direct funding of \$390,659. The initial twelve-month funding will require \$118,029, plus indirect costs of approximately \$36,590.

The cytotoxicity and carcinogenicity of asbestos and asbestos with adsorbed DMBA will be evaluated in rat and human respiratory epithelium in vitro. At various times after exposure exposed cell populations will be evaluated for altered growth capacity in vitro and for neoplastic transformations in vivo. These experiments a) will establish experimental procedures which can be applied in future in vitro studies of neoplastic development in human respiratory tissues following exposure to other potentially carcinogenic agents and b) will, for the first time, evaluate the carcinogenic effects of asbestos and DMBA directly on human respiratory tissues.

The following information is provided to assist in your review and approval of this proposal:

- a. This research effort is described in the approved ORNL Institutional Plan on page 52, "Work for Others (Excluding NRC)." The personnel required for this research are included in the direct FTE's in the "Summary of Resources" on page 53.
- b. M. Terzaghi-Howe will serve as the principal investigator devoting 100% of her effort to this research. Dr. Terzaghi-Howe currently devotes 100% effort to NCI (40-1044-79), which expires August 31, 1981.

40-1176-81
0 2/21

1078958

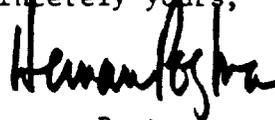
June 8, 1981

- c. Existing space and resources are available to conduct this research without interfering with existing DOE efforts.
- d. This research will serve the DOE related objectives in Activity HA 02 02 01 0, "Health Effects Research in Biomedical Systems - Carcinogenesis."

After your approval and approval of the Office of Health and Environmental Research, please forward six (6) copies to the Division of Research Grants, National Institutes of Health, Bethesda, Maryland 20205.

If you have any questions concerning this proposal, please contact M. Terzaghi-Howe, ext. 4-3971.

Sincerely yours,



Herman Postma
Director

HP:TV:ep

Enclosure

cc: J. E. Carr	W. R. Ragland
R. J. M. Fry	C. R. Richmond
R. A. Griesemer	M. Terzaghi-Howe
R. F. Hibbs	File - RC
J. N. Maddox, DOE-GTN	

1078959

DETACH AND CLIP TO THE SIGNED FACE PAGE OF THE APPLICATION

PERSONAL DATA ON
PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR

The Public Health Service has a continuing commitment to monitoring the operation of its review and award processes to detect—and deal appropriately with—any instances of real or apparent inequities with respect to age, sex, race, or ethnicity of the proposed principal investigator/program director.

To provide the PHS with the information it needs for this important task, the principal investigator/program director is requested to complete the form below and attach a single copy to the signed face page of the application.

Upon receipt and assignment of the application by the PHS, this form will be detached from the application. It will NOT be duplicated and will NOT be a part of the review process. Data will be confidential, and will be maintained in Privacy Act record system 09-25-0036, "Grants: IMPAC (Grant Contract Information)." All analyses conducted on the data will report aggregate statistical findings only and will not identify individuals.

If you decline to provide this information, it will in no way affect consideration of your application.

Your cooperation will be appreciated.

Date of Birth:
(Month/Day/Year)

Sex: Female Male

Race and/or Ethnic Origin:

Check one:

- American Indian or Alaskan Native
- Asian or Pacific Islander
- Black, not of Hispanic origin
- Hispanic
- White, not of Hispanic origin

NOTE: The category that most closely reflects the individual's recognition in the community should be used for purposes of reporting mixed racial and/or ethnic origins. Definitions are on the back of form.

1078960

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE GRANT APPLICATION FOLLOW INSTRUCTIONS CAREFULLY	LEAVE BLANK		
	TYPE	ACTIVITY	NUMBER
	REVIEW GROUP		FORMERLY
	COUNCIL BOARD (Month, year)		DATE RECEIVED

1. TITLE OF APPLICATION (Do not exceed 56 typewriter spaces)
 Neoplasia in human respiratory tissues - in vitro model

2. RESPONSE TO SPECIFIC PROGRAM ANNOUNCEMENT NO YES (If "YES," state RFA number and/or announcement title)

3. PRINCIPAL INVESTIGATOR / PROGRAM DIRECTOR

3a. NAME (Last, first, middle) 3b. SOCIAL SECURITY NUMBER
 Terzaghi-Howe, Margaret [REDACTED]

3c. MAILING ADDRESS (Street, city, state, zip code) 3d. POSITION TITLE
 Oak Ridge National Laboratory Staff Scientist
 Biology Division
 P.O. Box Y
 Oak Ridge, Tennessee 37830

3e. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT
 Biology Division

3f. TELEPHONE (Area code, number and extension) 3g. MAJOR SUBDIVISION
 (615)574-3971 Cancer and Toxicology Section

4. HUMAN SUBJECTS, DERIVED MATERIALS OR DATA INVOLVED 5. RECOMBINANT DNA RESEARCH SUBJECT TO NIH GUIDELINES
 NO YES (If "YES," form HHS 556 required) NO YES

6. DATES OF ENTIRE PROPOSED PROJECT PERIOD (This application) 7. TOTAL DIRECT COSTS REQUESTED FOR PROJECT PERIOD (from page 5) 8. DIRECT COSTS REQUESTED FOR FIRST 12-MONTH BUDGET PERIOD (from page 4)
 From: Dec. 1981 Through: Dec. 1984 \$ 390,659 \$ 118,029

9. PERFORMANCE SITES (Organizations and addresses) 10. INVENTIONS (Competing continuation application only)
 Biology Division Were any inventions conceived or reduced to practice during the course of the project?
 Oak Ridge National Laboratory NO YES - Previously reported
 P.O. Box Y YES - Not previously reported
 Oak Ridge, TN 37830

11. APPLICANT ORGANIZATION (Name, address, and congressional district)
 Oak Ridge National Laboratory
 P.O. Box Y
 Oak Ridge, TN 37830
 Third Congressional District

12. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR INSTITUTIONAL GRANT (See instructions)
 Code Description

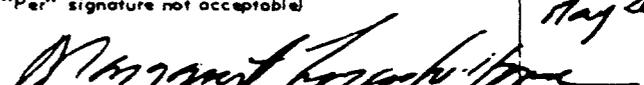
13. ENTITY IDENTIFICATION NUMBER
 60 - Department of Energy

14. TYPE OF ORGANIZATION (See instructions)
 Private Nonprofit
 Public (Specify Federal, State, Local):

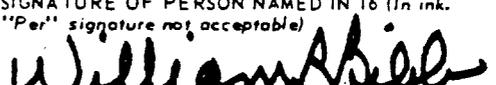
15. OFFICIAL IN BUSINESS OFFICE TO BE NOTIFIED IF AN AWARD IS MADE (Name, title, address and telephone number.)
 William R. Bibb
 Director, Research Division
 Department of Energy
 Oak Ridge, TN 37830
 FTS 626-0727, (615) 576-0727

16. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Name, title, address and telephone number)
 William R. Bibb
 Director, Research Division
 Department of Energy
 Oak Ridge, TN 37830
 FTS 626-0727, (615) 576-0727

17. PRINCIPAL INVESTIGATOR PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PERSON NAMED IN 3a (In ink. "Per" signature not acceptable) DATE
 May 20/81

18. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense. (U.S. Code, Title 18, Section 1001.)

SIGNATURE OF PERSON NAMED IN 16 (In ink. "Per" signature not acceptable) DATE
 7-8-81

1078961

ABSTRACT OF RESEARCH PLAN

NAME AND ADDRESS OF APPLICANT ORGANIZATION (Same as Item 11, page 1)
Oak Ridge National Laboratory, Biology Division,
P.O. Box Y, Oak Ridge, TN 37830

TITLE OF APPLICATION (Same as Item 1, page 1)
Neoplasia in human respiratory tissues - in vitro

Name, Title and Department of all professional personnel engaged on project, beginning with Principal Investigator/Program Director

M. Terzaghi Staff Scientist
 Biology Division
 Cancer and Toxicology

A. J. P. Klein-Szanto Medical Staff Scientist
 Biology Division
 Cancer and Toxicology

ABSTRACT OF RESEARCH PLAN: Concisely describe the application's specific aims, methodology and long-term objectives, making reference to the scientific disciplines involved and the health-relatedness of the project. The abstract should be self-contained so that it can serve as a succinct and accurate description of the application when separated from it. **DO NOT EXCEED THE SPACE PROVIDED.**

In the proposed experiments we will evaluate the cytotoxicity and carcinogenicity of asbestos alone and DMBA adsorbed to asbestos in rat tracheal epithelium and human respiratory epithelium. Human respiratory epithelium cultured in vivo on devitalized rat tracheal stroma implanted into the nude mouse and tracheal epithelium will be exposed in vitro and maintained in organ culture up to 4 wks. At various times after exposure tissues will be fixed and presence of preneoplastic lesions as well as changes in the fraction of ³HTdr labeled cells determined. At the same time intervals cells will be harvested from exposed explants and tested for the capacity to grow in soft agarose (EF assay) or yield tumors when implanted into nude mice. The carcinogenicity will be evaluated, if possible at equal dose levels and equitoxic dose levels in rat tissues and in the respiratory tissues of at least four different humans. Of interest are both interspecies and intraspecies (human) differences in cytotoxicity and carcinogenicity of asbestos and DMBA. Original contributions made by these experiments will be the (1) establishment of experimental procedures which can be applied in future in vitro studies of neoplastic development in human respiratory epithelium following exposure to other potentially carcinogenic agents and (2) direct evaluation of the effects of asbestos and DMBA on normal "cultured" human respiratory.

LABORATORY ANIMALS INVOLVED. Identify by common names. If none, state "none"

Fischer 344 rats and CC3-10 nude mice

TABLE OF CONTENTS

Number pages consecutively at the bottom throughout the application. Do not use suffixes such as 5a, 5b. Type the name of the Principal Investigator, Program Director at the top of each printed page and each continuation page.

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Face Page, Abstract, Table of Contents.....	1-3
Detailed Budget for First 12 Month Budget Period	4
Budget Estimates for All Years of Support.....	5
Biographical Sketch-Principal Investigator, Program Director (Not to exceed two pages).....	<u>7-8</u>
Other Biographical Sketches (Not to exceed two pages for each).....	<u>9-10</u>
Other Support.....	<u>11</u>
Resources and Environment	<u>12</u>
SECTION 2.	
Introduction (Excess pages; revised and supplemental applications)	_____
Research Plan	
A. Specific Aims (Not to exceed one page)	<u>15</u>
B. Significance (Not to exceed three pages).....	<u>15-18</u>
C. Progress Report/Preliminary Studies (Not to exceed eight pages)	<u>19-20</u>
D. Methods	<u>20-23</u>
E. Human Subjects, Derived Materials or Data.....	<u>24-26</u>
F. Laboratory Animals	_____
G. Consultants.....	_____
H. Consortium Arrangements or Formalized Collaborative Agreements	_____
I. Literature Cited	<u>27-29</u>
Checklist	<u>30</u>

SECTION 3. Appendix (Six sets) (No page numbering necessary for Appendix)

Number of publications: _____ Number of manuscripts: _____
 Other items (list): _____

Application Receipt Record, form PHS 3830
 Form HHS 596 if Item 4, page 1, is checked

1078963

DETAILED BUDGET FOR FIRST 12 MONTH BUDGET PERIOD DIRECT COSTS ONLY				FROM 12/1/81	THROUGH 11/30/82	DOLLAR AMOUNT REQUESTED (Omit cents)	
PERSONNEL (Applicant organization only) (See instructions)		TIME EFFORT		SALARY	FRINGE BENEFITS	TOTALS	
NAME	TITLE OF POSITION	%	Hours per Week				
M. Terzaghi-Howe	Principal Investigator	50	20	17,500 ^a	4,900	22,400	
A. Klein-Szanto		10	4	3,500 ^a	980	4,480	
Technician (to be named)	Technician	100	40	17,000 ^a	4,760	21,760	
SUBTOTALS					38,000	10,640	48,640
CONSULTANT COSTS (See instructions)							
None							
EQUIPMENT (Itemize)							
None							
SUPPLIES (Itemize by category)							
Animals and Animal Care ^c	6,877	Tissue culture supplies,					
EM and Histology Supplies		medium, carcinogen		14,000			
including photographic		Histology service ^d		3,000			
materials	4,000						
Glassware (Kitchen)	8,500						36,377
							800
TRAVEL		DOMESTIC					
		FOREIGN					
PATIENT CARE COSTS		INPATIENT					
		OUTPATIENT					
ALTERATIONS AND RENOVATIONS (Itemize by category)							
CONTRACTUAL OR THIRD PARTY COSTS (See instructions)							
OTHER EXPENSES (Itemize by category)							
Division Administration = \$11,400							
Publication costs = \$600							
\$13.50/sq. ft. laboratory space, utilities = \$17,212							
Maintenance and Service = \$3,000							32,212
TOTAL DIRECT COSTS (Also enter on page 1, item 8)							\$ 118,029

**BUDGET ESTIMATES FOR ALL YEARS OF SUPPORT REQUESTED
DIRECT COSTS ONLY**

BUDGET CATEGORY TOTALS		1st BUDGET PERIOD (from page 4)	ADDITIONAL YEARS SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL (Salary and fringe benefits.) (Applicant organization only)		48,640	53,500	58,850		
CONSULTANT COSTS		---				
EQUIPMENT		---				
SUPPLIES		36,377	40,015	44,017		
TRAVEL	DOMESTIC	800	880	968		
	FOREIGN	---				
PATIENT CARE COSTS	INPATIENT	---				
	OUTPATIENT	---				
ALTERATIONS AND RENOVATIONS						
CONTRACTUAL OR THIRD PARTY COSTS		---				
OTHER EXPENSES		32,212.	35,430	38,970		
TOTAL DIRECT COSTS		118,029 ^b	129,825 ^b	142,805 ^b		

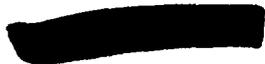
TOTAL FOR ENTIRE PROPOSED PROJECT PERIOD (Also enter on page 1, item 7) \longrightarrow \$ 390,659

JUSTIFICATION (Use continuation pages if necessary): Briefly describe the specific functions of the personnel and consultants. For all years, justify any costs for which the need may not be obvious, such as equipment, foreign travel, alterations and renovations, and contractual or third party costs. For future years, justify any significant increases in any category. In addition, for COMPETING CONTINUATION applications, justify any significant increases over current level of support. If a recurring annual increase in personnel costs is anticipated, give percentage.

^a ORNL uses the Uniform Cost Accounting System recommended by the Federal Cost Accounting Standards Board. This system makes use of Cost Collect Centers in which salaries of scientific and technical personnel performing similar tasks are averaged; associated costs (fringe benefits and Biology Division Administration) are calculated as percentages (28% and 30%, respectively) of that average cost.

Indirect costs have been established at a rate of 35% through an agreement negotiated between the NIH and the DOE. Because this percentage covers only part of costs that are often included by other institutions as indirect charges and because the DOE requires recovery of full costs, certain charges (utilities and Division Administration) are listed as Direct Costs on this form (shown under "Other Expenses").

^b The three year budget is calculated on the basis of an annual increase of 10% in expenses.



Justification (cont.):

^c Animals: The animals are bred and housed in a specific-pathogen-free facility, a requirement for precision research in respiratory carcinogenesis. Animal costs are calculated on a cage basis no matter how many animals per cage; however, the standard number in experimental cages is 5 rats/cage or 10 mice/cage. The cost unit in the Biology Division is MCE, i.e. "mouse cage unit", and 1 rat cage = 2 MCE. The SPF animal facilities are an integral part of the Respiratory Carcinogenesis Unit; therefore, cost for animals used for experiments must include the cost of maintaining the animal line, breeding cages, and animals held in stock until proper age (8-12 weeks old). The stock-holding number is determined by the anticipated needs of the investigators. It is important to note, therefore, that costs must represent some steady-state level with numbers of animals being used constantly and equivalent numbers entering from the breeding colony. The animal costs incurred also include those used fairly rapidly for making tracheal implants and finally the experimental cages which are long-term holdings. A proper breakdown of changes determined from long-term management of these facilities is to charge an additional 70% of the cost of the experimental and used animals for breeding and stock rats and to charge 200% for breeding and stock nude mice. The nude mice are more costly since the litters are a mixture of "nudes" and "hairys."

The projected animal cost per average MCE for March 1981 is \$14.60/month. This cost is determined from utilities, supplies, disease testing, manpower expenses (animal management, veterinarian, caretakers). It should be noted that fringe benefits and Biology Division overhead are included in these costs. The average estimated needs and cost of the experiments per year are:

SPF Rats

	Rats	MCE x #mos x cost/mo.	= Total cost
Use		25 x 3 x 14.60	= \$1,095
Breeding		18 x 12 x 14.60	= 3,154

Nude Mice

	Mice	MCE	
Use		5 x 12 x 14.60	= 876
Breeding		10 x 12 x 14.60	= <u>1,752</u>

Cost per year = \$6,877

^d Histology Service: Most of our material will be prepared as continuous sections of tracheal explants to identify lesions. Slides are charged on cost per "slide equivalent" basis. We anticipate an average of 1.2 slide equivalents/slide at a cost of \$6.34 per slide equivalent, 400 slides=\$3,043.

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Terzaghi-Howe, M.	Staff Scientist	[REDACTED]

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent to this application. Do not exceed 2 pages.

Experience

Undergraduate NSF Fellowship, Institute of Molecular Biology, University of Oregon, 1963. Supervisor, Prof. Franklin Stahl. Project in phage genetics.
 Teaching Assistant, Boston University, 1969.
 Research Assistant, Peter Bent Brigham Hospital, 1968.
 Research Assistant, Cancer and Toxicology Program, Biology Division, Oak Ridge National Laboratory, 1975-present.

Honors

[REDACTED]

Publications

Terzaghi, M. Master's Thesis, Boston University. Abscopal effects of radiation in hamster cheek pouch tumors. (Data presented at Radiation Research Meeting) 1971.

Terzaghi, M. and J. B. Little. Interactions between radiation and benzo(a)pyrene in an in vitro model for malignant transformation. In: Experimental Lung Cancer. Carcinogenesis and Bioassays (E. Karbe and J. Park, Eds.), Springer-Verlag, 1974.

McGandy, R. B., A. R. Kennedy, M. Terzaghi, and J. B. Little. Experimental respiratory carcinogenesis: Interaction between alpha-radiation and benzo(a)pyrene in the hamster. In: Experimental Lung Cancer. Carcinogenesis and Bioassays (E. Karbe and J. Park, Eds.), Springer-Verlag, 1974.

Terzaghi, M. Doctoral Thesis, Harvard University. Radiation-induced oncogenic transformation in mammalian cells. 1974.

Terzaghi, M. and J. B. Little. Repair of potentially lethal radiation damage in mammalian cells is associated with enhancement of malignant transformation. Nature 253: 548-549, 1975.

Terzaghi, M. and J. B. Little. Characterization of a hamster lung adenocarcinoma and its tissue culture adapted lines. J. Natl. Cancer Inst. 55, 1975.

Terzaghi, M. and J. B. Little. Oncogenic transformation in vitro: Influence of repair processes. In: The Biology of Radiation Carcinogenesis. Raven Press, 1975.

Terzaghi-Howe, M. [REDACTED]

Terzaghi Publications (continued)

Terzaghi, M. and J. B. Little. Radiation-induced transformation in a C3H mouse embryo derived cell line. *Cancer Res.* 36: 1367-1374, 1976.

Terzaghi, M. and J. B. Little. Oncogenic transformation in vitro following split-dose X-irradiation. *Int. J. Rad. Biol.* 29: 583, 1976.

Kennedy, A. R., A. Desrosiers, M. Terzaghi, and J. B. Little. Morphometric and histological analysis of the lungs of Syrian Golden hamsters. *J. Anatomy* 125: 527-553, 1978.

Nettesheim, P., A. C. Marchok, and M. Terzaghi. In vivo-in vitro studies of epithelial neoplasia. In: Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment, Vol. 2, pp. 307-329 (P. Ts'o and H. V. Gelboin, Eds.), Academic Press, 1978.

Terzaghi, M. In vivo-in vitro studies with respiratory tract epithelium exposed to carcinogen. II. Methods for defining altered behavior of epithelial cell populations. In: In Vitro Carcinogenesis. Guide to the Literature, Recent Advances and Laboratory Procedures (U. Saffiotti and H. Autrup, Eds.). NCI Technical Report Series No. 44, 1978.

Terzaghi, M., P. Nettesheim and M. L. Williams. Repopulation of denuded tracheal grafts with normal, preneoplastic and neoplastic epithelial cell populations. *Cancer Res.* 38: 4546-4553, 1978.

Terzaghi, M. and P. Nettesheim. The dynamics of neoplastic development in carcinogen exposed tracheal mucosa. *Cancer Res.* 39: 4003-4010, 1979.

Terzaghi, M. and A. J. P. Klein-Szanto. Differentiation of normal and cultured pre-neoplastic tracheal epithelial cells in rats. Importance of epithelial and mesenchymal interactions. *J. Natl. Cancer Inst.* 65: 1039-1048, 1980.

Terzaghi, M., P. Nettesheim, T. Yarita, and M. L. Williams. Epithelial focus assay for early detection of carcinogen altered cells in various organs of rats exposed in situ to N-nitrosoheptamethyleneimine. *J. Natl. Cancer Inst.* (submitted).

Klein-Szanto, A. J. P., B. Pal, M. Terzaghi and A. Marchok. Heterotopic tracheal transplants: Techniques and applications. In: Pulmonary Toxicology (G. Hook, Ed.), Raven Press, New York (in press).

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator / Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Andres J. P. Klein-Szanto	Medical Staff Scientist	[REDACTED]

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent to this application. Do not exceed 2 pages.

- 1966-1968: Instructor of Pathology, Department of Pathology, University of Buenos Aires, School of Medicine
- 1968-1974: Chief Instructor, Department of Pathology, University of Buenos Aires, Schools of Medicine and Dentistry
- 1970-1974: Staff Scientist, Pathology Section AEC, Buenos Aires
- 1974-1976: Oberassistent, Department of Structural Biology, University of Zurich
- 1976-1978: Head, Pathology Section, Argentine AEC, Buenos Aires
- 1978-present: Medical Staff Scientist, Oak Ridge National Laboratory, Biology Division

Selected Publications (from a total of 63 articles in refereed journals and books):

Unidirectional scanning for the microspectrophotometric investigation of enzyme reactions in squamous epithelium. R. L. Cabrini, A. J. P. Klein-Szanto, and M. E. Itoiz, Acta Histochem. 36:399-403, 1970.

Microspectrophotometric study of oxidative enzymes in irradiated epidermis. A. J. P. Klein-Szanto and R. L. Cabrini. Internatl. J. Radiat. Biol. 18(3):235-241, 1970.

Microspectrophotometric study of histoenzymic reactions in rat epidermis subjected to 0.5 Mrads of deuteron radiation. R. L. Cabrini, M. E. Itoiz, J. Mayo, E. E. Smolko, and A. J. P. Klein-Szanto. Internatl. J. Radiat. Biol. 18(5):414-421, 1970.

An oncogenic virus carried by hamster kidney cells. J. Mayo, J. H. Lombardo, A. J. P. Klein-Szanto, C. Conti, and J. L. Moriera. Cancer Res. 33:2273-2277, 1973.

Ultramorphological features of an experimental hamster sarcoma. A. J. P. Klein-Szanto, C. Conti, and J. Mayo. Z. Krebsforsch. 80:277-284, 1973.

Ultrastructure of irradiated nuclei: A. J. P. Klein-Szanto, B. M. de Rey, C. Conti, and R. L. Cabrini. Strahlentherap. 147:263-270, 1974.

Enzyme variations produced in epidermis by soft X-ray collimated beams. A. J. P. Klein-Szanto, R. L. Cabrini, M. E. Itoiz, and H. E. Volco. Int. J. Radiat. Biol. 26:285-288, 1974.

Microspectrophotometric study of acid phosphatase activity in irradiated squamous epithelium. M. E. Itoiz, C. Frasc, H. E. Volco, and A. J. P. Klein-Szanto. Strahlentherap. 147:643-648, 1974.

Metaplastic conversion of the differentiation pattern in oral epithelium affected by leukoplakia simplex. A stereologic study. A. J. P. Klein-Szanto, J. Banoczy, and H. E. Schroeder. Pathol. Eur. 11:189-210, 1976.

Terzaghi-Howe, M.

- Graded alterations of the epithelial differentiation pattern in buccal mucosa affected by lichen planus. A. J. P. Klein-Szanto, L. Andersen, and H. E. Schroeder. *Virchows Arch. Cell Pathol.* 22:245-261, 1976.
- Stereologic baseline data of normal human epidermis. A. J. P. Klein-Szanto. *J. Investig. Derm.* 68:73-78, 1977.
- Architecture and density of connective tissue papillae of the human oral mucosa. A. J. P. Klein-Szanto and H. E. Schroeder. *J. Anat. (Lond.)* 123:93-109, 1977.
- Clear and dark basal epithelial cells in human epidermis. A stereologic study. A. J. P. Klein-Szanto. *J. Cutan. Pathol.* 4:275-281, 1977.
- Ultrastructural stereology of irradiated epidermis. B. M. de Rey, A. J. P. Klein-Szanto, and R. L. Cabrini. *Radiation Res.* 77:103-116, 1979.
- Ultrastructural characteristics of carcinogen induced non-dysplastic changes in tracheal epithelium. A. J. P. Klein-Szanto, D. C. Topping, C. A. Heckman, and P. Nettesheim. *Am. J. Pathol.* 98:61-82, 1980.
- Ultrastructural characteristics of carcinogen-induced dysplastic changes in tracheal epithelium. A. J. P. Klein-Szanto, D. C. Topping, D. A. Heckman, and P. Nettesheim. *Am. J. Pathol.* 98:83-100, 1980.
- Studies on the mechanism of action of antitumor promoting agents: Their specificity in two-stage promotion. T. J. Slaga, A. J. P. Klein-Szanto, S. M. Fischer, C. E. Weeks, K. Nelson, and S. Major. *Proc. Natl. Acad. Sci. USA* 77:2251-2254, 1980.
- Sexual differences in the distribution of epithelial alterations in vitamin A deficient rats. A. J. P. Klein-Szanto, J. N. Clark, and D. H. Martin. *Int. J. Vitam. Nutr. Res.* 50:61-69, 1980.
- Cutaneous manifestations in rats with advanced vitamin-A deficiency. A. Klein-Szanto, D. H. Martin, and A. H. Pine. *J. Cutan. Pathol.* 7:260-270, 1980.
- Studies of neoplastic development in respiratory tract epithelium. P. Nettesheim, A. J. P. Klein-Szanto, A. C. Marchok, V. E. Steele, M. Terzaghi, and D. C. Topping. *Archiv. Pathol.* 105:1-10, 1981.
- Reestablishment of a mucociliary epithelium in tracheal organ cultures exposed to retinyl acetate - A biochemical and morphometric study. J. N. Clark, A. J. P. Klein-Szanto, A. H. Pine, K. B. Stephenson, and A. C. Marchok. *Europ. J. Cell Biol.* 21:261-268, 1980.
- In vivo differentiation of normal and cultured preneoplastic tracheal epithelial cells. The importance of epithelial mesenchymal interactions. M. Terzaghi and A. J. P. Klein-Szanto. *J. Natl. Cancer Inst.* 65:1039-1048, 1980.
- Induction of dark keratinocytes by 12-O-tetradecanoyl-13-phorbol-acetate and mezerein as an indicator of tumor-promoting efficiency. A. J. P. Klein-Szanto, S. K. Major, and T. J. Slaga. *Carcinogenesis* 1:399-406, 1980.
- Quantitative analysis of disturbed cell maturation in dysplastic lesions of the respiratory epithelium. A. J. P. Klein-Szanto, P. Nettesheim, D. C. Topping, and A. Olson. *Carcinogenesis* 1:1007-1016, 1980.
- Distribution and ultrastructural characteristics of dark cells in squamous metaplasias of the respiratory tract epithelium. A. J. P. Klein-Szanto, P. Nettesheim, A. Pine, and D. Martin. *Am. J. Pathol.* (in press).
- Modulation of growth, differentiation and mucous glycoprotein synthesis by retinyl acetate in cloned carcinoma cell lines. A. C. Marchok, J. N. Clark, and A. J. P. Klein-Szanto. *J. Natl. Cancer Inst.* (in press).
- Dark epithelial cells in preneoplastic lesions of the human respiratory tract. A. J. P. Klein-Szanto, P. Nettesheim, and G. Saccomanno (submitted).
- Skin tumor promoting activity of benzoyl peroxide, a widely used free radical generating compound. T. J. Slaga, A. J. P. Klein-Szanto, L. Triplett, L. Yotti, and J. E. Trosko. *Science* (in press).
- Experimental models for the study of lung cancer morphogenesis. In: *Morphogenesis of Lung Cancer*. Eds., Y. Shimosato, M. Melamed, and P. Nettesheim. P. Nettesheim and A. J. P. Klein-Szanto, CRC Press, Boca Raton (in press).
- Heterotopic tracheal transplants: Techniques and applications. In: *Pulmonary Toxicology*. Ed., G. Hook. A. J. P. Klein-Szanto, B. Pal, M. Terzaghi, and A. Marchok, Raven Press, NY, (in press).

OTHER SUPPORT
(USE CONTINUATION PAGES IF NECESSARY)

For each of the professionals named on page 2, list, in three separate groups: (1) active support; (2) applications pending review and/or funding; (3) applications planned or being prepared for submission. Include all Federal, non-Federal, and institutional grant and contract support. If none, state "NONE." For each item give the source of support identifying number, project title, name of principal investigator/program director, time or percent of effort on the project by professional named, annual direct costs, and entire period of support. (If part of a larger project, provide the titles of both the parent grant and the subproject and give the annual direct costs for each.) Briefly describe the contents of each item listed. If any of these overlap, duplicate, or are being replaced or supplemented by the present application, justify and delineate the nature and extent of the scientific and budgetary overlaps or boundaries.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: M. Terzaghi-Howe

(1) ACTIVE SUPPORT:

- A. NCI No. Y01-CP-90211. Dynamics of neoplastic development in the respiratory system.
 PI: M. Terzaghi. Effort: 85%
 Entire Period of Support: 9/79 - 8/81.
 Annual Direct Costs: 269,500.
- B. DOE Seed Money No. 3203-0214. In vivo Model for Chemical Carcinogenesis Studies Using Xenotransplanted Normal Human Tissues.
 PI: M. Terzaghi and A. Klein-Szanto Effort: 15%

(2) PENDING SUPPORT: NIH CA30271-01. Sensitivity of Initiated Cells to Carcinogenic Insults. Effort: 50%. Direct annual costs: 145,375.

(3) APPLICATION PLANNED: Neoplasia in human respiratory tumors. In vitro model

A. Klein-Szanto

(1) ACTIVE SUPPORT:

- A. NIEHS Interagency Agreement (40-662-77). Environmental Carcinogenesis Studies.
 PI: A. Klein-Szanto Effort: 80% Entire Period of Support: 6/80 - 6/84.
 Direct Annual Cost (6/81-6/82): 132,700.
- B. DOE Seed Money No. 3203-0214. Effort 10% (See above)
- C. NCI No. Y01-CP-90211. Effort 10% (See above)

(2) PENDING SUPPORT:

NIH CA-29556-01. Importance of Dark Cells in Skin Carcinogenesis.
 PI: A. Klein-Szanto Effort: 25%
 Annual Direct Costs: 61,250

A reduction in the effort on the NIEHS Interagency agreement is foreseen in order to invest that effort in the pending and the presently submitted grant proposals.

(3) SUBMITTING: None

RESOURCES AND ENVIRONMENT

FACILITIES: Mark the facilities to be used and briefly indicate their capacities, pertinent capabilities, relative proximity and extent of availability to the project. Use "other" to describe facilities at other performance sites listed in Item 9, page 1, and at sites for field studies. Using continuation pages if necessary, include a description of the nature of any collaboration with other organizations and provide further information in the RESEARCH PLAN.

- Laboratory:** A tissue culture laboratory will be used in this project and a histopathology laboratory (equipped with microtomes, cryotomes, ultramicrotomes, ovens).
- Clinical:**
- Animal:** Included in the Respiratory Carcinogenesis Unit is a modern barrier facility designed and operated to maintain rodents free of pathogens. It contains 12 holding rooms and a service area. The rodent production and holding rooms are operated on a strict barrier basis.
- Computer:** Computer facilities are available to the Biology Division.
- Office:** Office space adjacent to the laboratories.
- Other (_____):** Carcinogen preparation room - an appropriately vented room with weighing equipment set aside for carcinogen preparation only.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location, and pertinent capabilities of each.

Histopathology: Hitachi HU11B electron microscope, cryotomes, LKB Ultratome III-fluorescence microscope

Tissue culture: 72" open faced hood, 72" sterigard hood, three double door CO₂ incubators, inverted microscopes.

ADDITIONAL INFORMATION: Provide any other information describing the environment for the project. Identify support services such as consultants, secretarial, machine shop, and electronics shop, and the extent to which they will be available to the project.

Secretarial assistance is paid from indirect costs.

Maintenance and service are available upon request and are paid by means of direct costs.

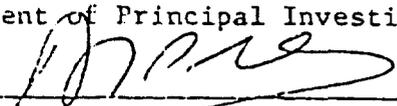
ORAU/ORNL COMMITTEE ON HUMAN STUDIES

Title of Proposal: REPOPULATION OF NORMAL HUMAN EPITHELIAL CELLS IN TRACHEAL TRANSPLANTS

Proposal No.: ORNL-6 Principal Investigator: Andres J. P. Klein-Szanto

Date of Approval: 4/18/80 Date of Disapproval: _____

Acknowledgment of Principal Investigator:



Signature

5-14-80
Date

Approval for Proposal ORNL-6 was granted by the Committee on Human Studies; Dr. Klein-Szanto is (1) to make certain that the hospital pathology departments have a consent form noting that tissues can and will be used for "other scientific purposes," (2) that the consent form submitted to the Committee with Proposal ORNL-6 is to be used as a model, and (3) that a proper consent form from the hospital pathology departments be filed with the proposal using the same words as given in Section 2 of the submitted "model" consent form.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

GRANT CONTRACT FELLOW OTHER

PROTECTION OF HUMAN SUBJECTS
ASSURANCE/CERTIFICATION/DECLARATION

NEW RENEWAL CONTINUATION
APPLICATION IDENTIFICATION NUMBER (If known)

ORIGINAL FOLLOWUP REVISION

STATEMENT OF POLICY: Safeguarding the rights and welfare of subjects at risk in activities supported under grants and contracts from DHEW is primarily the responsibility of the institution which receives or is accountable to DHEW for the funds awarded for the support of the activity. In order to provide for the adequate discharge of this institutional responsibility, it is the policy of DHEW that no activity involving human subjects to be supported by DHEW grants or contracts shall be undertaken unless the Institutional Review Board has reviewed and approved such activity, and the institution has submitted to DHEW a certification of such review and approval, in accordance with the requirements of Public Law 93-348, as implemented by Part 46 of Title 45 of the Code of Federal Regulations, as amended, (45 CFR 46). Administration of the DHEW policy and regulation is the responsibility of the Office for Protection from Research Risks, National Institutes of Health, Bethesda, Md 20014.

1. TITLE OF PROPOSAL OR ACTIVITY

Neoplasia in Human Respiratory Tissues - In Vitro Model

2. PRINCIPAL INVESTIGATOR ACTIVITY DIRECTOR/FELLOW

Terzaghi, Margaret

3. DECLARATION THAT HUMAN SUBJECTS EITHER WOULD OR WOULD NOT BE INVOLVED

A. NO INDIVIDUALS WHO MIGHT BE CONSIDERED HUMAN SUBJECTS, INCLUDING THOSE FROM WHOM ORGANS, TISSUES, FLUIDS, OR OTHER MATERIALS WOULD BE DERIVED, OR WHO COULD BE IDENTIFIED BY PERSONAL DATA, WOULD BE INVOLVED IN THE PROPOSED ACTIVITY. (IF NO HUMAN SUBJECTS WOULD BE INVOLVED, CHECK THIS BOX AND PROCEED TO ITEM 7. PROPOSALS DETERMINED BY THE AGENCY TO INVOLVE HUMAN SUBJECTS WILL BE RETURNED.)

B. HUMAN SUBJECTS WOULD BE INVOLVED IN THE PROPOSED ACTIVITY AS EITHER: NONE OF THE FOLLOWING, OR INCLUDING: MINORS, FETUSES, ABORTUSES, PREGNANT WOMEN, PRISONERS, MENTALLY RETARDED, MENTALLY DISABLED. UNDER SECTION 6. COOPERATING INSTITUTIONS, ON REVERSE OF THIS FORM, GIVE NAME OF INSTITUTION AND NAME AND ADDRESS OF OFFICIAL(S) AUTHORIZING ACCESS TO ANY SUBJECTS IN FACILITIES NOT UNDER DIRECT CONTROL OF THE APPLICANT OR OFFERING INSTITUTION.

(will use autopsy material obtained from designated pathology service)

4. DECLARATION OF ASSURANCE STATUS/CERTIFICATION OF REVIEW

A. THIS INSTITUTION HAS NOT PREVIOUSLY FILED AN ASSURANCE AND ASSURANCE IMPLEMENTING PROCEDURES FOR THE PROTECTION OF HUMAN SUBJECTS WITH THE DHEW THAT APPLIES TO THIS APPLICATION OR ACTIVITY. ASSURANCE IS HEREBY GIVEN THAT THIS INSTITUTION WILL COMPLY WITH REQUIREMENTS OF DHEW Regulation 45 CFR 46, THAT IT HAS ESTABLISHED AN INSTITUTIONAL REVIEW BOARD FOR THE PROTECTION OF HUMAN SUBJECTS AND, WHEN REQUESTED, WILL SUBMIT TO DHEW DOCUMENTATION AND CERTIFICATION OF SUCH REVIEWS AND PROCEDURES AS MAY BE REQUIRED FOR IMPLEMENTATION OF THIS ASSURANCE FOR THE PROPOSED PROJECT OR ACTIVITY.

B. THIS INSTITUTION HAS AN APPROVED GENERAL ASSURANCE (DHEW ASSURANCE NUMBER G1716) OR AN ACTIVE SPECIAL ASSURANCE FOR THIS ONGOING ACTIVITY, ON FILE WITH DHEW. THE SIGNER CERTIFIES THAT ALL ACTIVITIES IN THIS APPLICATION PROPOSING TO INVOLVE HUMAN SUBJECTS HAVE BEEN REVIEWED AND APPROVED BY THIS INSTITUTION'S INSTITUTIONAL REVIEW BOARD IN A CONVENED MEETING ON THE DATE OF 4-18-80 IN ACCORDANCE WITH THE REQUIREMENTS OF THE Code of Federal Regulations on Protection of Human Subjects (45 CFR 46). THIS CERTIFICATION INCLUDES, WHEN APPLICABLE, REQUIREMENTS FOR CERTIFYING FDA STATUS FOR EACH INVESTIGATIONAL NEW DRUG TO BE USED (SEE REVERSE SIDE OF THIS FORM).

THE INSTITUTIONAL REVIEW BOARD HAS DETERMINED, AND THE INSTITUTIONAL OFFICIAL SIGNING BELOW CONCURS THAT:

EITHER HUMAN SUBJECTS WILL NOT BE AT RISK; OR HUMAN SUBJECTS WILL BE AT RISK.

3. AND 5. SEE REVERSE SIDE

7. NAME AND ADDRESS OF INSTITUTION

Oak Ridge National Laboratory
P.O. Box Y
Oak Ridge, TN 37830

8. TITLE OF INSTITUTIONAL OFFICIAL

Richard A. Griesemer, Biology Division Director

TELEPHONE NUMBER

(615)574-0213

9. SIGNATURE OF INSTITUTIONAL OFFICIAL

DATE

HEW-505 (Rev. 4-75)

ENCLOSE THIS FORM WITH THE PROPOSAL OR RETURN IT TO REQUESTING AGENCY.

1078974

I. Specific Aims

A. To adapt organ and cell culture techniques to the study of neoplastic development in human bronchial or tracheal epithelial cells "cultured" in vivo. We have recently developed techniques whereby human respiratory epithelial cells can be cultured in vivo on frozen-thawed rat tracheal stroma implanted into the nude mouse. Various cell culture and organ culture techniques developed in our laboratory and others will be adapted to the study of neoplastic development in human epithelium cultured in this manner.

B. To evaluate the toxic and carcinogenic effects of crocidolite, chrysotile or amosite asbestos with and without adsorbed carcinogen (DMBA) on human and rat respiratory epithelium in vitro. Cytotoxicity will be evaluated in histologic preparations of exposed tissues maintained in organ culture up to 4 weeks post-exposure. Carcinogenic effects will be evaluated 1) by means of the EF assay in vitro, and 2) by histologic evaluation of tissues xenotransplanted into the nude mouse 4 weeks after exposure.

C. To evaluate differences in the cytotoxicity and carcinogenicity of asbestos and asbestos-DMBA in bronchial and/or tracheal tissues obtained from at least four different donors.

D. To evaluate difference between the cytotoxicity and carcinogenicity of asbestos and asbestos-DMBA to human and rat respiratory epithelium.

II. Background and Significance

A vast majority of clinically important human neoplasms are epithelial in origin. Epidemiologic data suggest that a majority of these neoplasms are caused by carcinogenic and/or promoting agents present in the environment (1). There is a clear need for experimental models involving direct evaluation of the effects of carcinogenic and promoting agents on human epithelial cells. For the most part, experiments are carried out with human (e.g. 2) or rodent fibroblasts (e.g. 3) in culture or in rodent tissues in vivo (e.g. 4, 5) or in culture (e.g. 6,7,8). It is often impossible to extrapolate from these tissues to human epithelial cells. The capacity of these cell populations to metabolize carcinogens is often quantitatively different from that observed in human epithelial cells (9,10). Agents identified as being toxic and/or carcinogenic in humans have been found to be innocuous or less effective in rodents (11,12). In our discussion of the background and significance of the proposed experiments we will focus on the following questions. 1) What technical problems have made direct experimentation with normal human respiratory tissues difficult? 2) What experimental approaches have been developed for studying neoplastic development in rodent respiratory tissues? What are the advantages and disadvantages of each for purposes of carrying out similar studies with human respiratory epithelium? 3) What data have been reported which would permit one to extrapolate knowledge about the carcinogenic process in rodents or human fibroblasts to human epithelial cell populations? What experimental data are required to make valid interspecies comparisons? 4) In summary, how will the experiments herein proposed a) fulfill the need for experimentation with normal human respiratory tissues, b) relate to existing rodent models developed for respiratory carcinogenesis studies, and c) make valid interspecies comparisons.

The principal obstacles encountered when attempting to carry out experiments with normal human respiratory epithelial cells have been 1) the problems involved with obtaining normal human tissues and 2) the inability to propagate normal human respiratory epithelial cells. For technical reasons most experiments with human tissues have been carried out in either normal human fibroblast cultures (e.g. 2,13, 14) or in fibroblasts obtained from individuals with a genetic disease predisposing them to cancer (e.g. 15,16). Dermal fibroblasts are readily available from most hospital obstetric services and can also be readily grown in culture. Tissues from internal organs such as the lung can only be obtained at time of surgery for e.g. tuberculosis or cancer, or from immediate autopsies. Tissues from patients with respiratory diseases or from adults previously exposed to a variety of environmental pollutants may not be normal. Ideally, respiratory tissues should be obtained from genetically normal newborns who die for reasons not related to neoplastic disease. Because relatively small amounts of tissue can be obtained from any single donor, it is then essential to develop those techniques required for propagation of these tissues. Only then can extensive carcinogenesis experiments be carried out with cells from a single donor.

A number of experimental techniques have been developed in order to study neoplastic development in rodent respiratory tissues. These in general have included a) the in vivo approach, b) organ culture, and c) the in vivo approach combined with cell or organ culture. These techniques will be reviewed from the perspective of developing effective protocol for studying neoplastic development in human respiratory tissues.

The tracheal transplant has been developed in our laboratory as a model for studying the neoplastic development in rat tracheal epithelium (4,17). The essential features of this model are the following: a) Intact tracheas are transplanted subdermally. Exposure is carried out by insertion of a pellet containing a measured amount of particulate, carcinogen, or promoter which is then released over a fixed interval of time (4,18). b) At any time following exposure, tracheas can be removed and evaluated for morphologic alterations. In this way, one can study those morphologic changes which precede neoplastic development. This approach has been effectively employed in studies of neoplastic development in rat tracheal epithelium exposed to DMBA (19), BP (4), particulates (20), or promoter (21). Several disadvantages should be noted. First, it is expensive to carry out in vivo experiments. Second, one can only infer from the data which morphologic changes ultimately give rise to neoplastic lesions. Because histologic evaluation involves destruction of the tissue one cannot ascertain the ultimate fate of the cells comprising the lesion.

Organ cultures of both human (1) and rodent (8) respiratory tissues have been extensively used in carcinogenesis studies. Small segments of tracheal or bronchial tissue are placed into an appropriate culture medium. Tissues derived from human, rat and hamsters can then be maintained in organ culture for weeks (1,5,22). Carcinogen (1,22) or particulates (23,24) are applied to the tissue explant which is then evaluated histologically, at various times after exposure, for the development of toxic or "preneoplastic" changes. Explant cultures of human and rodent tissues offer several advantages. 1) One can expose various cell types in the intact organized tissue to chemicals or particulates. 2) One can observe histopathologic changes in the tissue following exposure in the absence of host influences, a

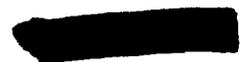
factor which appears to be of critical importance in studies involving carcinogen exposure of human (preliminary data). 3) They are less costly than in vivo experiments.

Organ cultures have proven to be extremely valuable for the study of carcinogen toxicity and carcinogen metabolism in respiratory tissues (1,22). However, the value of this model for purposes of studying neoplastic transformation induced by chemical carcinogens alone appears to be limited. Efforts to induce neoplastic transformation in rodent and human respiratory tissues exposed to chemicals alone have not been successful (22,23). However, it has been reported that exposure of hamster tracheal explants to carcinogen adsorbed to particulates (asbestos or FeO₃) has been reported to induce neoplastic transformation (20). This may be due to either the continual release of carcinogen from the particulate which has been incorporated into the tracheal mucosa or due to a cocarcinogenic effect of the particulate (23,24). This approach has not been employed in studies with human respiratory tissues.

A combined in vivo and in vitro approach has been developed in our laboratory whereby neoplastic development in vivo in rat tracheal mucosa can be studied by means of cell culture techniques (6,7). Exposure is carried out in vivo by insertion of a carcinogen pellet into the tracheal transplant. At various times after exposure, tracheas are removed and cell cultures (7,17) established. In these experiments, it has been shown that shortly after carcinogen exposure, cells which have a markedly increased growth capacity in vitro can be isolated from the exposed tissues. Cell populations isolated from normal nonexposed tracheas rarely continue to proliferate more than one to four weeks in cell culture (7,25). This approach has made it possible to quantitate in vitro the emergence, in vivo, of carcinogen-altered and neoplastic cell populations after exposure to DMBA (see EF-assay, under section on preliminary data). The EF assay appears to be a sensitive tool for detecting cells with neoplastic potential in carcinogen exposed tissues (7). A tumor induction study in which tracheal transplants were exposed, over a 4 week period to 165 µg DMBA showed an incidence of 9% invasive tracheal carcinomas. In contrast, cell populations isolated in culture demonstrated that at 8 months 80% of the tracheas contained cells with neoplastic potential. Thus, such an approach might increase the probability of detecting neoplastic cells in human tissues which may be refractory to the induction of neoplastic transformation.

Over the past decades, great strides have been made toward understanding neoplastic disease by using, for example, rodent tissues as an experimental model. It is clearly important to evaluate the relevance of these data to the development of human cancers. Toward this end, a number of experiments have been carried out. Experiments carried out with human fibroblasts suggest that relative to, for example, rodent fibroblasts, human fibroblasts are difficult to transform in culture (26). Fibroblasts from individuals with a genetic predisposition for developing neoplastic disease are more readily transformed in culture (16,26). Normal human fibroblasts may be particularly refractory to the induction of neoplastic transformation in that they appear to have a significantly lower carcinogen metabolizing capability than human epithelial cells (9,27). In general, the pathways used for metabolism of various chemicals is similar; however, the rate of metabolism is often quite different (10).

Two experiments have been reported which involve exposure of xenotransplanted segments of human bronchus to large doses of DMBA (28) or MBA (29). The tumor incidences are very low in comparison to those reported in similar experiments with rat trachea (19). Despite the efforts of a number of laboratories, little definitive information is available on interspecies differences in carcinogenesis.



It is clearly important to develop techniques whereby normal human and rodent target tissues can be studied in parallel under comparable experimental conditions.

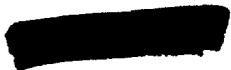
Epidemiologic studies suggest a link between asbestos exposure and an increased incidence of bronchogenic and pulmonary carcinomas in humans (30,31). A number of studies have been carried out in experimental animals in order to gain some insight into the role of asbestos in the induction of respiratory neoplasias (e.g. 20,23,31). With the exception of preliminary data reported by Haugen et al. (32), the role of asbestos as a cocarcinogen/carcinogen has not been evaluated directly in human respiratory tissues.

There is an unresolved controversy as to whether asbestos acts as a cocarcinogen or complete carcinogen for the induction of human respiratory neoplasias.

Summary:

The experiments herein described adapt various cell culture techniques previously developed for the study of neoplastic development in rodent tissues to the study of human cells propagated in vivo in the nude mouse. The rationale for each phase of these experiments is summarized below.

1. The lack of experimental carcinogenesis data with human respiratory epithelium has stemmed mainly from the inability to propagate and expand populations of human epithelial cells. We have developed techniques whereby human respiratory epithelium can be propagated in vivo on devitalized rat tracheal stroma implanted into the nude mouse. In this way, extensive carcinogenesis studies can ultimately be carried out with normal respiratory epithelium obtained from each individual fetal or newborn donor. This has not heretofore been possible.
2. Asbestos and asbestos-DMBA will be employed in these experiments for the following reasons. a) Asbestos has been implicated in the causation of human respiratory neoplasias. b) DMBA has been extensively used in in vivo experiments with rodent respiratory tissues. This background information will be important during the initial phases of the proposed experiments and perhaps lead to a more effective analysis of data. c) Neither human nor rodent respiratory tissues have yielded neoplastic lesions following exposure to chemical carcinogens in culture. However, organ cultures of rodent tracheas exposed to asbestos with adsorbed carcinogen have developed neoplastic lesions. Thus, our chances of inducing neoplastic transformation in vitro appears to be greatly enhanced if asbestos is used in combination with chemical carcinogen.
3. Both rat tracheas and reconstituted human bronchi or tracheas will be exposed and maintained in organ culture for up to 4 weeks after exposure. In this way, we hope to a) minimize host-cell interactions which might interfere with early expression of the neoplastic phenotype and b) minimize cost.
4. The EF assay will be used to monitor sequential changes in the growth potential of epithelial cells exposed to carcinogen. This will be carried out a) in order to quantitate the emergence of cell populations with different in vitro growth capacities, and b) to maximize the possibility of detecting cells with neoplastic potential. The latter may be particularly important for the studies with human tissues which could be relatively resistant to the induction of neoplastic transformation.



III. Progress/Preliminary Data

A. Epithelial focus (EF) assay

Over the past several years we have developed a technique referred to as the epithelial focus assay which enables us to assess in vitro, qualitative and quantitative cellular changes presumed to occur in tracheal epithelium in vivo during the process of carcinogenesis (7,25). Viable epithelial cells are harvested from heterotopic tracheal grafts immediately or up to one year after exposure in vivo for 4 weeks to total doses of 5-300 μ g of DMBA. The cell suspensions obtained from individual tracheas were seeded into culture dishes and the frequency of proliferating epithelial foci (EF) determined one month later. Normal non-exposed tracheal cells yield few EF in culture. In contrast, 80-100% of tracheas exposed to DMBA yielded 10 to 100 times more EF per trachea. Three different types of EF could be distinguished experimentally: EF which could not be subcultured (EF₀), EF which could be subcultured but did not grow in soft agarose (EF_{s,ag-}), and those EF which could be subcultured and grown in soft agarose (EF_{s,ag+}). Our findings suggest the existence, in the epithelium of carcinogen exposed tracheas, of EF with different in vitro growth capacities. No carcinogen-dose effect on EF frequency has been observed when tracheas are exposed to 5-300 μ g DMBA for 2-4 weeks. No EF were detected following 1 wk exposure to 20 μ g DMBA. Thus, the length of exposure rather than total dose appears to be of major importance. With increased carcinogen dose and increased time in vivo after exposure, there is an increase in the frequency of subculturable EF which also grow in agar. The EF assay will be used during the course of proposed experiments to monitor those changes in in vitro growth characteristics which occur at various times following exposure of human and rat respiratory epithelium in organ culture.

B. "In vivo culture" and carcinogen exposure of human respiratory epithelium

We have recently reported that human cells will survive and differentiate into a normal appearing mucociliary epithelium on frozen-thawed rat tracheal stroma implanted into the nude mouse (36). Human newborn or fetal respiratory tissues obtained at autopsy are harvested enzymatically and inoculated into frozen-thawed rat tracheas and implanted subdermally in the scapular region of the nude mouse. Within 2 to 3 weeks a normal appearing mucociliary epithelium is formed. Epithelial cells (5-10 x 10⁵) can then be stripped from each repopulated trachea and seeded into a series of 3 to 5 new frozen-thawed rat tracheas in order to expand the pool of reconstituted human tracheas. We have to date succeeded in subpassaging 5 different human cell lines from separate individuals up to 6 times in vivo. These cells do not survive in cell culture. Preliminary work, involving for example, chromosome analysis and collection of preliminary carcinogen dose data in human respiratory epithelium cultured in vivo is currently being carried out with DOE seed money funds. A normal human diploid karyotype has been found in tracheal epithelial cells passaged several times in vivo. At this point we do not know how many times human respiratory epithelial cells can be passaged and still yield a mucociliary epithelium in vivo and a normal diploid karyotype. Survival, morphology and karyotype will be assessed at each subsequent passage.

"Cultured" human bronchial and tracheal epithelium have been exposed to DMBA (75-250 μ g) released from beeswax pellets into the reconstituted tracheal lumen over a period of four weeks. Exposed tissues were evaluated morphologically at 2, 4, and 8 weeks after exposure and cells harvested for the EF assay immediately or four weeks after exposure. An intense toxic reaction was noted following exposure to 250 μ g of DMBA. This included partial ulceration of the epithelium and an intense inflammatory response involving massive infiltration of host (nude mouse) macrophages. This

response was less marked immediately following exposure to 75 µg of DMBA and four weeks following exposure to 250 µg DMBA. Ulcerated epithelium was ultimately replaced by hyperplastic or metaplastic epithelium with orthokeratinized epidermoid metaplasias predominating. Using the same culture conditions previously used in studies of rat tracheal epithelium, no EF were isolated immediately following exposure to 250 µg of DMBA. By four weeks following exposure to 250 µg up to 75% of the tracheas contained 2 to 40 EF. Immediately following exposure to 75 µg of DMBA 25% of tracheas yielded 2 to 4 EF. To date, no EF isolated from control non-exposed tracheas have been subculturable. However, several EF isolated from carcinogen exposed tracheas have been subculturable. These data suggests that the EF assay previously developed in our laboratory for studies of neoplastic development in rat tracheal epithelium will also be a useful tool for evaluating in vitro, neoplastic development in human respiratory epithelium.

IV. Methods

A. General

1. Intact reconstituted human or rat tracheas will be exposed in vitro to HBSS alone, asbestos in HBSS, or asbestos-DMBA in HBSS.
2. Organ cultures will be established from control and exposed tissues.
3. At 24 hours after exposure segments of trachea will be digested and the amount of ³H-DMBA absorbed into tissue explants measured.
4. At 24 hours, 72 hours, 1 week, 2 weeks, and 4 weeks tissue segments will be fixed for morphologic evaluation.
5. At 1, 2, and 4 weeks postexposure organ cultures will be placed into "outgrowth cultures." These cells will then be harvested enzymatically and tested for subculturability and growth in soft agarose (EF assay).
6. At 4 weeks, organ culture explants will be implanted into nude mice.

B. Animals

Rat tracheas will be obtained from female Fischer 344 rats 8 to 10 weeks of age. Nude mice to be used for xenotransplantation will be CC3-10 females. Animals are all bred and housed in our own specific-pathogen-free facility. Prior to performing surgical procedures, animals are anesthetized by metophane inhalation.

C. Human Tissues

At least four different human bronchial and/or tracheal epithelial cell populations will be cultured in vivo. Tissues are received on ice within 48 hours of autopsy of a newborn or human fetus. Medical history and cause of death will be known and only those individuals with no known genetic abnormalities will be used. Tissues will be cut into roughly 0.5 cm² pieces and placed in complete Hams F-12 medium and maintained at 37° in a 5% CO₂ atmosphere for roughly 7 days. During this period of time, viable cell populations on each tissue segment divide such that the entire

surface is covered with viable mucociliary epithelium (33). At the end of this incubation time, cells are stripped from each tissue segment by incubation in pronase (1% in PBS) for 20 minutes at 37° with occasional agitation. The resulting cell suspension, consisting mainly of epithelial cells mixed most likely with a small number of fibroblasts, is inoculated into each intact 2x frozen-thawed (FT) rat trachea (37). Both ends of each inoculated trachea are tied off with surgical silk to prevent leakage of inoculated cells and implanted subdermally into nude mice (2 tracheas per mouse). Within 2 to 3 weeks a normal human tracheal or bronchial mucosa is established. Human cells can then again be harvested enzymatically and seeded into a new series of 3-5 FT tracheas in order to amplify the number of "human tracheas." In this manner "human tissues" are available for the proposed experiments plus have a reserve for future experiments. Tracheas to be used in the proposed experiments will be removed from the host mouse 3 or more weeks after cell inoculation and implantation when a normal epithelium has been established.

At this time we do not know how long one can passage human epithelial cell in vivo with a normal morphology and karyotype. At this time we have successfully passaged cells 6 times. All experiments will be carried out at a passage level which has previously been determined to be usable in that tracheas contain epithelial cells which are judged to be morphologically and karyotypically normal.

D. Exposure

Carcinogens and particulates will be prepared as follows. The carcinogen, DMBA, will be dissolved in acetone. Asbestos, amosite, crocidolite, or chrysotile (UICC standard reference samples) suspended in cold acetone will be added to the DMBA and agitated on a magnetic stirrer for 48 hours. Carcinogen coated asbestos will be allowed to settle and residual acetone removed under nitrogen. The preparation will then be washed twice with distilled water. Modifications of this procedure have been previously described (23,34). All carcinogens and particulates will be handled in an appropriately vented carcinogen hood.

Exposure of tracheal tissues will be carried out in vitro by inoculation of HBSS alone (controls) or HBSS containing either asbestos alone or asbestos-DMBA into the intact tracheal lumen. Tracheas will be sealed off with surgical silk to prevent leakage. During exposure (2 to 6 hours) tracheas will be maintained at 37°C in a 5% CO₂ atmosphere. Following exposure, tracheas will be rinsed with HBSS to remove unabsorbed particulate.

In order to estimate the dose of DMBA to the epithelium, tissues will be exposed to asbestos with adsorbed DMBA (1:100, 1:1000 ³H-DMBA:cold DMBA). At 24 hours after exposure tissues will be digested with Protosol and the amount of tritium labeled DMBA in each segment of organ culture counted in a scintillation counter. Total DMBA per trachea will then be calculated from ³H-DMBA standard curves. Advice, when required, will be obtained from Dr. J. Selkirk, Biology Division, ORNL. The amount of asbestos penetrating into tracheal epithelial cells will be evaluated by SEM and/or TEM 24 hours after exposure.

Methods chosen for exposure of tissue may be found to be unacceptable because normal tissue cannot be maintained in a normally differentiated, viable state under the conditions required for adequate exposure. At this stage, we don't know how long tissues must be exposed in order to deliver a reproducible and

effective (nontoxic yet carcinogenic) dose to both human and rat respiratory epithelium. Craighead et al. (23) report effective results following 1 hour exposures of hamster organ culture explants to carcinogen absorbed to asbestos. Haugen et al. (32) have used 6 hours for exposure of human bronchial organ cultures. If only 1 to 2 hours are required for effective exposure, most likely no problems will be encountered. If, however, 6 hours or more are required, normal tissues may be adversely affected. Other methods to be tried, in the event that the proposed techniques are inadequate, are those described by Craighead et al. (23) involving exposure of small segments of trachea in organ culture or that of Topping et al. (20) involving exposure of intact tracheal transplants in vivo by insertion of a gelatin pellet containing asbestos or asbestos-DMBA.

The doses of DMBA and the type of asbestos to be used in these experiments will be determined in a series of preliminary experiments. Effective dose levels will initially be determined on the basis of the cytotoxic effects of the treatments involved. In preliminary experiments we will evaluate the cytotoxicity of chrysotile, crocidolite or amosite of asbestos over a dose range of 1 to 1000 μg per ml (20,32) delivered over a period of 1-6 hrs. The type and dose of asbestos to be finally employed in carcinogenesis studies will be determined on the basis of toxicity (determined morphologically) and the capacity to induce "preneoplastic" lesions in vitro. Chrysotile asbestos has been observed to be 10 fold more toxic than either amosite or crocidolite and may be too toxic to use effectively in these experiments (32). Exposure of hamster tracheal organ cultures to crocidolite asbestos initially induces changes similar to those induced by promoters (24). Carcinomas developed later. For these reasons crocidolite asbestos might yield the most interesting results and will be the form of asbestos most extensively tested in preliminary experiments. Initially, we will aim to deliver DMBA doses ranging from 5 to 300 μg per trachea. This dose range has been previously studied in rat tissues both in vivo and in vitro. Again, toxicity and induction of preneoplastic lesions will be evaluated. Doses to be employed in more extensive studies will be chosen on the basis of these criteria.

E. Organ cultures

Organ cultures will be established following exposure in vitro of intact reconstituted human trachea and/or bronchi and rat tracheas. Each trachea will be cut in half longitudinally and divided transversely into 5 equal portions. Organ cultures will be maintained in complete Ham's F12^a in a 5% CO₂ atmosphere. Tissue segments will be maintained in shallow medium such that the tissue is only partially submerged. The medium chosen for maintenance of organ culture should be adequate to maintain normal cell differentiation in both rat and human tissues for 4 weeks. It is possible that human tissue explants will not survive or remain normally differentiated in Hams F12^a. In this case, we will employ those culture conditions previously described by Harris et al. (1). A chamber gassed with 50% O₂ and 45% CO₂ maintained at 36.5°C is rocked at 10 cycles per minute. The medium of choice would be CMRL 1066 supplemented with glutamine, insulin, hydrocortisone, retinyl acetate, penicillin, streptomycin, plus 5% heat inactivated fetal calf serum (1).

F. Morphologic evaluation of organ culture explants

In order to evaluate toxicity, 4 tissue explants per exposure group will be fixed in Bouin's 24 hours, 72 hours, one week, two weeks and four weeks after

^aComplete Hams F-12: Hams F-12 plus 5% fetal bovine serum (FBS, organ cultures) or 10% FBS (cell culture), insulin (0.1 $\mu\text{g}/\text{ml}$), hydrocortisone (0.1 $\mu\text{g}/\text{ml}$), penicillin (100 Iu/ml), streptomycin (100 $\mu\text{g}/\text{ml}$), and if required β -retinyl acetate (0.1 $\mu\text{g}/\text{ml}$).

exposure for histologic evaluation. Prior to fixation, organ explants will be labeled with $^3\text{HTdR}$ for 5 hrs. (35). Tissues to be evaluated at the light microscope level will be fixed in Bouin's solution and embedded in paraffin and sections stained with hematoxylin and eosin. Sections to be used for autoradiography will be dipped in emulsion and later developed. For electron microscopy, tissues will be fixed in 3% glutaraldehyde in 0.05 M 2,1,6 trimethylpyridine buffer post-fixed in 2% osmic acid in 0.1 M cacodylate buffer, dehydrated, and embedded in Epon 812. Ultrathin sections will be stained with uranyl acetate and lead citrate and observed in a Hitachi HB11 electron microscope. The following criteria will be employed for purposes of evaluating asbestos and asbestos-DMBA induced changes in respiratory epithelium. a) The presence of cell degeneration and sloughing with no cell regeneration as measured by the frequency of $^3\text{HTdR}$ labeled cells will be considered extreme toxicity. b) The induction of hyperplasias, metaplasias, and dysplasias will be interpreted as suggestive of "preneoplasia." Changes in the proliferative capacity of exposed and normal cells with increased time in organ culture will be evaluated by noting changes in the frequency of $^3\text{HTdR}$ labeled cells in fixed tissues and by their capacity to yield outgrowths when placed in "outgrowth" culture (see below).

G. Testing for malignant transformation

1. Outgrowth cultures and EF assay

At 1, 2 and 4 weeks after exposure tissue explants will be placed in plastic tissue culture dishes containing complete Hams F12^a (7). Under these conditions, cells migrate from the tissue explant onto the tissue culture dish (6). When 1 cm² outgrowths are established, explants will be sequentially moved ("replanted") in order to establish up to 5 outgrowths per explant. Cells from each explant will be harvested enzymatically (0.2% trypsin-EDTA/CMF Hanks plus 5% chick serum incubated for 7 minutes at 37°C) and the EF assay carried out as follows: a) Cells are counted in a hemacytometer and seeded into triplicate 60 mm tissue culture dishes at densities of 10³ to 5 x 10⁴ cells per dish. b) One month after seeding cells the number of proliferating epithelial foci (EF) per 10⁴ cells is determined. c) EF are isolated and passaged into separate tissue culture dishes. d) Subculturable EF are tested for growth in soft agarose.

If no isolated EF are found to grow in soft agarose with the protocol described above, two alternative paths will be taken. First, isolated EF will be cultured in vitro and tested for growth in soft agarose at a later time (7). Second, changes in media conditions such as decreases in the concentration of calcium will be used. Lowered calcium levels reportedly increase the probability of isolating neoplastic cells from exposed human tissues (14,38). Third, cells may possibly need to be left in primary culture for longer periods of time (months) prior to the development of cells with oncogenic potential (8).

2. Xenotransplantation

At one and four weeks after exposure, 8 explants from each experimental group will be implanted subdermally to x-irradiated (400 rads) nude mice (2 explants/mouse). Implants will be palpated weekly in order to detect developing tumors. All explants not developing tumors will be removed one year after exposure and fixed for histologic (light or EM) evaluation. The presence of carcinoma in situ, metaplastic or dysplastic lesions and evidence of tissue rejection will be noted.

H. Data (Human and Rat Tissues)

1. Controls

In order to determine the suitability of those conditions chosen for exposure and maintenance of organ cultures, tissues will be exposed to HBSS for 2, 4, or 6 hrs. Tracheas will then be divided into two equal halves lengthwise and each half divided transversely into five equal pieces, thus yielding 10 explants/trachea.

Explants from each exposure group will be maintained for up to 28 days. At four day intervals, eight explants per group will be harvested and evaluated as follows: a) Four explants will be placed in outgrowth culture to evaluate the proliferative capacity of control tissues. b) If explants above yield outgrowths, four explants will be evaluated morphologically and % ³HTdR labeled cells determined.

Data will be expressed in terms of % explants yielding epithelial cell outgrowths and rate of outgrowth (increased diameter of outgrowth with time). Conditions causing explants to yield no outgrowths will be considered unacceptable. If either < 4 days exposure combined with < 20 days in organ culture are excessively toxic to control tissues of either species, alternative conditions will be similarly tested.

2. Crocidolite asbestos - toxicity

Doses ranging from 1-1000 µg/ml and exposure intervals ranging from 1-6 hours will initially be used. Toxicity will be evaluated at 1 day, 3 days, 1, 2, and 4 weeks after exposure as described above.

Those doses which completely inhibit the outgrowth of epithelial cells will be considered excessively toxic. Again, morphologic evaluation will only be carried out if viable outgrowths are observed.

3. DMBA - asbestos toxicity

At this time, we do not know how much DMBA adsorbs to asbestos nor how much actually gets to the cell from the asbestos. A wide range of concentrations of DMBA will initially be mixed with the crocidolite asbestos. Explants will be exposed 1-6 hrs to various dose levels of DMBA adsorbed to amounts of asbestos which were previously found to be either non-toxic or marginally toxic (50% of explants + outgrowths). Toxicity of DMBA-asbestos will be evaluated as described above under "controls." Morphologic evaluation will be carried out only if outgrowths are observed in \geq 50% of explants.

4. Dosimetry

Explants will be exposed to 3 dose levels of DMBA-asbestos or asbestos previously determined to be either non-toxic, marginally toxic (75% explants yield outgrowths) or toxic (25% explants yield outgrowths). When possible, equal doses as well as doses which are equitoxic to both human and rat tissues will be used. Doses actually delivered to the tissues will be determined as follows.

- a) asbestos: The frequency of fibers penetrating epithelial cells will be determined at the light microscope level and/or by TEM or SEM. Four explants/dose group will be evaluated.
- b) DMBA: A mixture of 3/4 DMBA and cold DMBA will be adsorbed to asbestos. At 24 hours post-exposure, explants (8/group) will be digested with protosol and ^3H -DMBA measured. Total DMBA ($\mu\text{g} \pm \text{Sd}$)/ explant will then be calculated from standard ^3H -DMBA curves using least-squares fits of the logs technique.

5. EF assay

The same dose levels used above ("Dosimetry") for human and rat tissues will be employed. At 1, 2 and 4 weeks following exposure, 4-8 explants (depending on toxicity) will be placed in outgrowth culture. Outgrowths will be subcultured and subculturable outgrowths tested for growth in soft agarose in order to determine a) EF frequency, b) % EF which are subculturable, and c) % subculturable EF which grow in soft agarose.

Significant differences between dose groups and between species (equitoxic and equal dose levels) will be established by means of the t-test (EF frequency) and χ^2 -test (% EFs and EFs, ag+). Variation between explants within the same dose group, and between dose groups will be measured.

6. Xenotransplantation

At 4 weeks following exposure, 8 explants/dose group will be implanted into x-irradiated nude mice (4/mouse). Explants will be removed when lesions 1 cm^2 are palpated. Explants not yielding tumors within one year will be harvested and fixed for histologic evaluation.

Data will be evaluated in terms of frequency (%) of explants yield tumors, time to tumor development (range and average \pm sd). In explants not yielding tumors the frequency of explants with normal mucociliary epithelium, metaplasias and dysplasias with moderate to severe atypias will be scores. These data will be analyzed by means of the χ^2 test.

I. Tentative Schedule

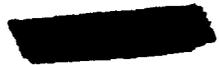
Year 1

- (1) Determine suitability of proposed methods for exposure and maintenance of human and rat tissues.
- (2) Evaluate toxicity of asbestos and determine which form is most suitable for the proposed experiments.
- (3) Evaluate toxicity of DMBA-asbestos and determine what dose levels are suited for use in the proposed experiment.

Year 2

- (1) Expose rat tracheas and those established from two separate human donors.
- (2) Evaluate exposed tissues for preneoplastic and neoplastic alterations in vivo and in vitro.

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Year 3

- (1) Expose at least two more pools of human respiratory epithelium.
- (2) Evaluate tissues in vivo and in vitro for the appearance of preneoplastic or neoplastic alterations.
- (3) If time permits, tissues will be similarly exposed to, for example, BP adsorbed to asbestos and evaluated in vivo and in vitro.

J. Personnel

Dr. A. J. P. Klein-Szanto will be responsible for morphologic evaluation of tissues. Dr. M. Terzaghi-Howe will be responsible for all other aspects of the project involving exposure, dosimetry, and culture of the cells.

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CHECKLIST

This is the required last page of the application.

Check the appropriate boxes and provide the information requested.

TYPE OF APPLICATION:

- NEW application (This application is being submitted to the PHS for the first time.)
- COMPETING CONTINUATION of grant number: _____
(This application is to extend a grant beyond its original project period.)
- SUPPLEMENT to grant number: _____
(This application is for additional funds during a funded project period.)
- REVISION of application number: _____
(This application replaces a prior version of a new, competing continuation or supplemental application.)
- Change of Principal Investigator/Program Director.
Name of former Principal Investigator/Program Director: _____

ASSURANCES IN CONNECTION WITH:

Civil Rights	Handicapped Individuals	Sex Discrimination	Human Subjects General Assurance (if applicable)	Laboratory Animals (if applicable)
<input checked="" type="checkbox"/> Filed <input type="checkbox"/> Not filed				

INDIRECT COSTS:

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office. If the applicant organization is in the process of initially developing or renegotiating a rate, or has established a rate with another Federal agency, it should, immediately upon notification that an award will be made, develop a tentative indirect cost rate proposal based on its most recently completed fiscal year in accordance with the principles set forth in the pertinent DHHS Guide for Establishing Indirect Cost Rates, and submit it to the appropriate DHHS Regional Office. Indirect costs will not be paid on foreign grants, construction grants, and grants to individuals, and usually not on grants in support of conferences.

- DHHS Agreement Dated: February 14, 1979
_____ % Salary and Wages or 35 % Total Direct Costs.

Is this an off-site or other special rate, or is more than one rate involved? YES NO

Explanation: _____

- DHHS Agreement being negotiated with _____ Regional Office.
- No DHHS Agreement, but rate established with _____ Date _____
- No Indirect Costs Requested.