137701

00041

RECEIVED
OCT 7 1985
U.S. EPA REGION 7
WASTE MANAGEMENT DIVISION
HAZARDOUS WASTE COORDINATOR

ENVIRONMENTAL HEALTH PERSPECTIVES

Volume 53, November 1983

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

David P. Rall, Director,
National Institute of Environmental Health Sciences

EDITORS

George W. Lucier

Gary E. R. Hook

BOARD OF EDITORS

John R. Bend
Colin F. Chignell
Robert L. Dixon
Hans L. Falk
Robert A. Goyer
Larry G. Hart
Joseph K. Haseman
David G. Hoel
James E. Huff
Burke Judd
Heinrich V. Malling
H. B. Matthews
Ernest E. McConnell
James D. McKinney
Clifford L. Mitchell
Warren T. Piver
Conrad B. Richter
Raymond W. Tennant

EDITORIAL REVIEW BOARD

Phillip W. Albro
John Bachmann
J. Carl Barrett
Stephen C. Bondy
Gary A. Boorman
Arnold R. Brody
Rajendra S. Chhabra
Gary Clemons
Terri Damstra
Jack H. Dean
Richard P. DiAugustine
Robert T. Drew
Thomas E. Eling
Richard B. Everson
Lawrence Fishbein
Bruce A. Fowler
Donald Gardner
Joe W. Grisham
Frank E. Guthrie
James R. Hass
Victor Hasselblad
Ernest Hodgson
Michael Hogan
David J. Holbrook
David G. Kaufman
Carole A. Kimmel
George M. Kingman
Albert C. Kolbye, Jr.
Kenneth S. Korach
Martin R. Krigman
Donald I. McRee
Tom S. Miya
Robert A. Neal
William C. Nelson
Richard M. Philpot
Herbert S. Posner
Robert M. Pratt
Jerry M. Rice
Walter J. Rogan
Seamus A. Rooney
Michael D. Shelby
William Sheridan
Robert E. Staples
Velimir B. Vouk
Michael D. Waters
Hanspeter Witschi
Errol Zeiger

EDITORIAL STAFF

Dorothy L. Ritter
Assistant Managing Editor

Ruth Krigman, Copy Editor
Linda Hein, Editorial Assistant

CONTENTS

Summary Workshop on Ingested Asbestos

J. R. Millette. Preface 1

L. W. Condie. Review of published studies of orally administered asbestos 3

E. E. McConnell, A. M. Shefner, J. H. Rust and J. A. Moore. Chronic effects of dietary exposure to amosite and chrysotile asbestos in Syrian golden hamsters 11

E. E. McConnell, H. A. Rutter, B. M. Ulland and J. A. Moore. Chronic effects of dietary exposure to amosite asbestos and tremolite in F344 rats 27

J. R. Millette, P. J. Clark, J. Stober and M. Rosenthal. Asbestos in water supplies of the United States 45

G. M. Marsh. Critical review of epidemiologic studies related to ingested asbestos 49

L. Polissar, R. K. Severson and E. S. Boatman. Cancer risk from asbestos in drinking water: summary of a case-control study in western Washington 57

E. E. Sigurdson. Observations of cancer incidence surveillance in Duluth, Minnesota 61

P. M. Conforti. Effect of population density on the results of the study of water supplies in five California counties 69

M. E. Tarter, R. C. Cooper and W. R. Freeman. A graphical analysis of the interrelationships among waterborne asbestos, digestive system cancer and population density 79

J. R. Millette, G. F. Craun, J. A. Stober, D. F. Kraemer, H. G. Tousignant, E. Hildago, R. L. Duboise and J. Benedict. Epidemiology study of the use of asbestos-cement pipe for the distribution of drinking water in Escambia County, Florida 91

L. S. Erdreich. Comparing epidemiologic studies of ingested asbestos for use in risk assessment 99

M. S. Kanarek. The San Francisco Bay epidemiology studies on asbestos in drinking water and cancer incidence: relationship to studies in other locations and pointers for further research 105

J. W. Meigs. Assessment of studies on cancer risks from asbestos in Connecticut drinking water 107

R. C. Cooper. Comments on the California studies 109

W. J. Nicholson. Human cancer risk from ingested asbestos: a problem of uncertainty 111

J. N. Rowe. Relative source contributions of diet and air to ingested asbestos exposure 115

P. M. Cook. Review of published studies on gut penetration by ingested asbestos fibers 121

E. S. Boatman, T. Merrill, A. O'Neill, L. Polissar and J. R. Millette. Use of quantitative analysis of urine to assess exposure to asbestos fibers in drinking water in the Puget Sound region 131

K. Seshan. How are the physical and chemical properties of chrysotile asbestos altered by a 10-year residence in water and up to 5 days in simulated stomach acid? 143

CONTENTS

Summary Workshop on Ingested Asbestos

J. R. Millette. Preface 1

L. W. Condie. Review of published studies of orally administered asbestos 3

E. E. McConnell, A. M. Shefner, J. H. Rust and J. A. Moore. Chronic effects of dietary exposure to amosite and chrysotile asbestos in Syrian golden hamsters 11

E. E. McConnell, H. A. Rutter, B. M. Ulland and J. A. Moore. Chronic effects of dietary exposure to amosite asbestos and tremolite in F344 rats 27

J. R. Millette, P. J. Clark, J. Stober and M. Rosenthal. Asbestos in water supplies of the United States 45

G. M. Marsh. Critical review of epidemiologic studies related to ingested asbestos 49

L. Polissar, R. K. Severson and E. S. Boatman. Cancer risk from asbestos in drinking water: summary of a case-control study in western Washington 57

E. E. Sigurdson. Observations of cancer incidence surveillance in Duluth, Minnesota 61

P. M. Conforti. Effect of population density on the results of the study of water supplies in five California counties 69

M. E. Tarter, R. C. Cooper and W. R. Freeman. A graphical analysis of the interrelationships among waterborne asbestos, digestive system cancer and population density 79

J. R. Millette, G. F. Craun, J. A. Stober, D. F. Kraemer, H. G. Tousignant, E. Hildago, R. L. Duboise and J. Benedict. Epidemiology study of the use of asbestos-cement pipe for the distribution of drinking water in Escambia County, Florida 91

L. S. Erdreich. Comparing epidemiologic studies of ingested asbestos for use in risk assessment 99

M. S. Kanarek. The San Francisco Bay epidemiology studies on asbestos in drinking water and cancer incidence: relationship to studies in other locations and pointers for further research 105

J. W. Meigs. Assessment of studies on cancer risks from asbestos in Connecticut drinking water 107

R. C. Cooper. Comments on the California studies 109

W. J. Nicholson. Human cancer risk from ingested asbestos: a problem of uncertainty 111

J. N. Rowe. Relative source contributions of diet and air to ingested asbestos exposure 115

P. M. Cook. Review of published studies on gut penetration by ingested asbestos fibers 121

E. S. Boatman, T. Merrill, A. O'Neill, L. Polissar and J. R. Millette. Use of quantitative analysis of urine to assess exposure to asbestos fibers in drinking water in the Puget Sound region 131

K. Seshan. How are the physical and chemical properties of chrysotile asbestos altered by a 10-year residence in water and up to 5 days in simulated stomach acid? 143

M. E. Meek. Transmigration of ingested asbestos 149

W. H. Hallenbeck. Asbestos penetration of the gastrointestinal tract 153

B. T. Mossman. *In vitro* approaches for determining mechanisms of toxicity and carcinogenicity by asbestos in the gastrointestinal and respiratory tracts 155

F. B. Daniel. *In vitro* assessment of asbestos genotoxicity 163

G. S. Logsdon. Engineering and operating approaches for controlling asbestos fibers in drinking water 169

P. Toft and M. E. Meek. Asbestos in drinking water: a Canadian view 177

J. A. Cotruvo. Asbestos in drinking water: a status report 181

G. M. Marsh. Additional thoughts on the review of epidemiologic studies related to ingested asbestos 185

L. Polissar, R. K. Severson and E. S. Boatman. Additional notes on the case-control study in western Washington on the cancer risk from asbestos in drinking water 189

P. M. Conforti. Additional comments on the study of the effect of population density on the results of the California Bay area study 191

M. E. Tarter. Answers to some questions raised by the presentation on the graphical analysis of the interrelationships between waterborne asbestos, digestive system cancer and population density 193

E. S. Boatman. Additional notes on the use of quantitative analysis of urine to assess exposure to asbestos fibers in drinking water in the Puget Sound region 195

K. Patel-Mandlik and J. Millette. Accumulation of ingested asbestos fibers in rat tissues over time 197

J. R. Millette. Summary of discussion sessions: workshop on ingested asbestos 201

Announcement 205

Published Volumes of EHP 207

Instructions to Authors 209

M. E. Meek. Transmigration of ingested asbestos 149

W. H. Hallenbeck. Asbestos penetration of the gastrointestinal tract 153

B. T. Mossman. *In vitro* approaches for determining mechanisms of toxicity and carcinogenicity by asbestos in the gastrointestinal and respiratory tracts 155

F. B. Daniel. *In vitro* assessment of asbestos genotoxicity 163

G. S. Logsdon. Engineering and operating approaches for controlling asbestos fibers in drinking water 169

P. Toft and M. E. Meek. Asbestos in drinking water: a Canadian view 177

J. A. Cotruvo. Asbestos in drinking water: a status report 181

G. M. Marsh. Additional thoughts on the review of epidemiologic studies related to ingested asbestos 185

L. Polissar, R. K. Severson and E. S. Boatman. Additional notes on the case-control study in western Washington on the cancer risk from asbestos in drinking water 189

P. M. Conforti. Additional comments on the study of the effect of population density on the results of the California Bay area study 191

M. E. Tarter. Answers to some questions raised by the presentation on the graphical analysis of the interrelationships between waterborne asbestos, digestive system cancer and population density 193

E. S. Boatman. Additional notes on the use of quantitative analysis of urine to assess exposure to asbestos fibers in drinking water in the Puget Sound region 195

K. Patel-Mandlik and J. Millette. Accumulation of ingested asbestos fibers in rat tissues over time 197

J. R. Millette. Summary of discussion sessions: workshop on ingested asbestos 201

Announcement 205

Published Volumes of EHP 207

Instructions to Authors 209

IN THE NEXT ISSUE

Metallothionein and Cadmium Nephrotoxicity Conference

L. FRIBERG. Cadmium and the kidney

M. NORDBERG. General aspects of cadmium-metallothionein in biological fluids

K. T. SUZUKI. Studies of cadmium uptake and metabolism by the kidney

K. S. SQUIBB. Intracellular metabolism and effects of circulating cadmium-metallothionein in the kidney following acute treatment

K. WAKU. The chemical form of cadmium in subcellular fractions following cadmium exposure

K. KOSTIAL. Effects of age/diet on cadmium uptake on renal cadmium deposition

M. R. S. FOX. Effect of diet and dietary forms of cadmium on renal deposition

S. ONOSAKA. Effects of other metals on tissue deposition of cadmium in metallothionein

D. H. PETERING. Effects of zinc deficiency on renal uptake of cadmium and chemical form of cadmium in subcellular fractions

N. REVIS. Dietary protein effects on cadmium absorption via the gastrointestinal tract and subsequent deposition in the kidney

J. H. R. KAGI. Structure of metallothioneins

M. KARIN. Human metallothionein genes: structure and function

D. R. WINGE. Role of metal clusters in cadmium binding during intracellular turnover

I. ARMITAGE. Metallothionein turnover and ^{113}cadmium binding in clusters

R. LAUWERYS. Characterization of cadmium proteinuria in rats/man

E. AUGHEY. Effect of cadmium on renal blood vessels

K. NOGAWA. Biologic indicators of cadmium nephrotoxicity in persons with low level cadmium exposure

Z. SHAIKH. Urinary cadmium-metallothionein as an indicator of cadmium nephrotoxicity

R. LILIS. Epidemiologic study of renal function in copper smelter workers

G. KAZANTZIS. Renal function in relation to low levels of cadmium exposure in a group of lead, zinc, and cadmium smelter workers

K. NOMIYAMA. Reversibility of cadmium nephropathy in primates

G. NORDBERG. Problems with chelation in cadmium poisoning

B. ENGSTROM. Effects of chelating agents on oral uptake and renal deposition and excretion of cadmium

C. D. KLAASSEN. Alteration of tissue deposition of cadmium by chelating agents

M. G. CHERIAN. Chelation of cadmium without renal damage

O. ANDERSON. Chelation of cadmium

Environmental Health Perspectives
Vol. 53, p. 1, 1983

Preface

The United States Environmental Protection Agency (U.S. EPA) has been concerned about the question of possible adverse health effects of asbestos fibers in drinking waters since the early 1970s. A number of research projects were initiated in epidemiology, toxicology, *in vitro* testing, engineering, and other areas to investigate as many aspects of this question as possible. On October 13 and 14, 1982, a workshop was held at the U.S. EPA Breidenbach Environmental Research Center in Cincinnati to summarize what had been learned from the work sponsored on ingested asbestos. The papers included in these proceedings consist of reviews performed by Agency personnel or contractors and presentations of the unpublished results of U.S. EPA sponsored projects. Two papers presented by the Department of National Health and Welfare, Ottawa, Ontario, Canada are also included. The primary focus of the workshop was to present scientific summaries and identify possible areas where further investigation might be useful. In general, policy issues were not discussed.

These proceedings have been subjected to the peer and policy review system of the U.S. EPA and approved for publication. The views and policies presented are those of the individual authors and do not necessarily reflect those of the U.S. EPA.

Each technical paper had the appraisal of at least two reviewers. The comments of the following researchers who served as reviewers are gratefully acknowledged: K. Adler, A. Bender, E. Boatman, D. Coffin, L. Condie, G. Craun, A. Dean, M. Dourson, L. Erdreich, P. Gartside, D. Greathouse, W. Hallenbeck, R. Hertzberg, I. Higgins, M. Kanarek, G. Kirmeyer, G. Logsdon, L. McCabe, P. McCauley, W. Meigs, L. Palekar, Y. Patel, L. Polissar, W. Smith, C. Sonich, R. Stevens, and J. Symons. S. Marshall of the Dynamac Corporation, Rockville, MD, is gratefully acknowledged for her support in organizing the conference and proceedings.

JAMES R. MILLETTE
Health Effects Research Laboratory
U.S. EPA
Cincinnati, OH 45268

SUMMARY WORKSHOP ON INGESTED ASBESTOS

October 13-14, 1982
Cincinnati, Ohio

Sponsored by the
U.S. Environmental Protection Agency

V. EYBL. Interaction of chelating agents with cadmium in mice

C. G. ELINDER. Metallothionein in rabbit kidneys preserved for transplantation

A. GLENNAS. Cadmium uptake and metabolism in cultured cells

S. G. JONES. Structure-activity relationships among dithiocarbamate antidotes for acute cadmium chloride intoxication

D. M. TEMPLETON. Physiochemical and metabolic properties of modified metallothioneins

K. A. MELIS. Crystals of cadmium, zinc metallothionein

Abstracts of Poster Sessions

Contributed Article

J. KALDOR. The statistical association between cancer incidence and major-cause mortality, and estimated residential exposure to air emissions from petroleum and chemical plants

SUMMARY WORKSHOP ON INGESTED ASBESTOS

October 13-14, 1982
Cincinnati, Ohio

Sponsored by the
U.S. Environmental Protection Agency

IN THE NEXT ISSUE

Metallothionein and Cadmium Nephrotoxicity Conference

L. FRIBERG. Cadmium and the kidney

M. NORDBERG. General aspects of cadmium-metallothionein in biological fluids

K. T. SUZUKI. Studies of cadmium uptake and metabolism by the kidney

K. S. SQUIBB. Intracellular metabolism and effects of circulating cadmium-metallothionein in the kidney following acute treatment

K. WAKU. The chemical form of cadmium in subcellular fractions following cadmium exposure

K. KOSTIAL. Effects of age/diet on cadmium uptake on renal cadmium deposition

M. R. S. FOX. Effect of diet and dietary forms of cadmium on renal deposition

S. ONOSAKA. Effects of other metals on tissue deposition of cadmium in metallothionein

D. H. PETERING. Effects of zinc deficiency on renal uptake of cadmium and chemical form of cadmium in subcellular fractions

N. REVIS. Dietary protein effects on cadmium absorption via the gastrointestinal tract and subsequent deposition in the kidney

J. H. R. KAGI. Structure of metallothioneins

M. KARIN. Human metallothionein genes: structure and function

D. R. WINGE. Role of metal clusters in cadmium binding during intracellular turnover

I. ARMITAGE. Metallothionein turnover and ^{113}cadmium binding in clusters

R. LAUWERYS. Characterization of cadmium proteinuria in rats/man

E. AUGHEY. Effect of cadmium on renal blood vessels

K. NOGAWA. Biologic indicators of cadmium nephrotoxicity in persons with low level cadmium exposure

Z. SHAIKH. Urinary cadmium-metallothionein as an indicator of cadmium nephrotoxicity

R. LILIS. Epidemiologic study of renal function in copper smelter workers

G. KAZANTZIS. Renal function in relation to low levels of cadmium exposure in a group of lead, zinc, and cadmium smelter workers

K. NOMIYAMA. Reversibility of cadmium nephropathy in primates

G. NORDBERG. Problems with chelation in cadmium poisoning

B. ENGSTROM. Effects of chelating agents on oral uptake and renal deposition and excretion of cadmium

C. D. KLAASSEN. Alteration of tissue deposition of cadmium by chelating agents

M. G. CHERIAN. Chelation of cadmium without renal damage

O. ANDERSON. Chelation of cadmium

V. EYBL. Interaction of chelating agents with cadmium in mice

C. G. ELINDER. Metallothionein in rabbit kidneys preserved for transplantation

A. GLENNAS. Cadmium uptake and metabolism in cultured cells

S. G. JONES. Structure-activity relationships among dithiocarbamate antidotes for acute cadmium chloride intoxication

D. M. TEMPLETON. Physiochemical and metabolic properties of modified metallothioneins

K. A. MELIS. Crystals of cadmium, zinc metallothionein

Abstracts of Poster Sessions

Contributed Article

J. KALDOR. The statistical association between cancer incidence and major-cause mortality, and estimated residential exposure to air emissions from petroleum and chemical plants

Environmental Health Perspectives
Vol. 53, p. 1, 1983

Preface

The United States Environmental Protection Agency (U.S. EPA) has been concerned about the question of possible adverse health effects of asbestos fibers in drinking waters since the early 1970s. A number of research projects were initiated in epidemiology, toxicology, *in vitro* testing, engineering, and other areas to investigate as many aspects of this question as possible. On October 13 and 14, 1982, a workshop was held at the U.S. EPA Breidenbach Environmental Research Center in Cincinnati to summarize what had been learned from the work sponsored on ingested asbestos. The papers included in these proceedings consist of reviews performed by Agency personnel or contractors and presentations of the unpublished results of U.S. EPA sponsored projects. Two papers presented by the Department of National Health and Welfare, Ottawa, Ontario, Canada are also included. The primary focus of the workshop was to present scientific summaries and identify possible areas where further investigation might be useful. In general, policy issues were not discussed.

These proceedings have been subjected to the peer and policy review system of the U.S. EPA and approved for publication. The views and policies presented are those of the individual authors and do not necessarily reflect those of the U.S. EPA.

Each technical paper had the appraisal of at least two reviewers. The comments of the following researchers who served as reviewers are gratefully acknowledged: K. Adler, A. Bender, E. Boatman, D. Coffin, L. Condie, G. Craun, A. Dean, M. Dourson, L. Erdreich, P. Gartside, D. Greathouse, W. Hallenbeck, R. Hertzberg, I. Higgins, M. Kanarek, G. Kirmeyer, G. Logsdon, L. McCabe, P. McCauley, W. Meigs, L. Palekar, Y. Patel, L. Polissar, W. Smith, C. Sonich, R. Stevens, and J. Symons. S. Marshall of the Dynamac Corporation, Rockville, MD, is gratefully acknowledged for her support in organizing the conference and proceedings.

JAMES R. MILLETTE
Health Effects Research Laboratory
U.S. EPA
Cincinnati, OH 45268

Environmental Health Perspectives
Vol. 53, pp. 3–9, 1983

Review of Published Studies of Orally Administered Asbestos

by Lyman W. Condie*

There has been great public concern about the adverse health effects resulting from the presence of asbestos fibers in municipal drinking water supplies. This article reviews and summarizes the experimental findings of 11 published papers that have evaluated the carcinogenic potential of asbestos following its ingestion. The long-term, high-level ingestion of various types of asbestos fibers in more than one animal species failed to produce any definite, reproducible, organ-specific carcinogenic effect.

Introduction

Prolonged industrial human exposure to asbestos has been associated with an increase in the incidence of certain forms of cancer. The relationship between inhaled asbestos and mesotheliomas of the pleura and peritoneum or pulmonary carcinoma is particularly strong; other cancers have also been implicated from inhaled asbestos (*1–3*). In order to explain why cancer may occur at remote sites following the inhalation of asbestos, it has been suggested that the asbestos fibers that are cleared from the lungs are swallowed and subsequently migrate through the gastrointestinal wall to the peritoneum where cancer may be initiated.

Originally the question of pathogenicity from asbestos exposure had relevance only to people occupationally exposed, but the discovery of amphibole fibers in municipal water supplies (*4*) indicated that asbestos was more widely dispersed through the environment than once believed. Asbestos from natural sources, as well as from mining activities, has been shown to contaminate bodies of water that are used as sources of drinking water. Asbestos fibers have been detected in commercial beverages, possibly resulting from the use of asbestos filters. The extensive use of asbestos cement pipe in municipal water systems has concerned officials of the U.S. Environmental Protection Agency. This paper summarizes the various published asbestos ingestion studies that have attempted to answer the question of whether or not ingested asbestos is a health hazard. Other relevant topics such as gastrointestinal penetration by asbestos, the presence of asbestos in municipal drinking water supplies, and epidemiologic studies will be presented in subsequent papers of this workshop. The details of all but one of the cited experimental studies are summarized in Table 1.

Results of Published Studies

An abstract by Bonser and Clayson (*5*) initially reported experimental findings from an ingestion study. Asbestos was administered to Sprague-Dawley rats in their feed at a level of 0.15%. No malignant tumors were observed in the exposed animals, which may have been due to the low level of asbestos administered. The high mortality of the rats due to pulmonary infection seriously compromised the study.

Webster reported the only study conducted with primates (*6*). Because of lack of experimental detail, the findings of this article were omitted from Table 1. An unreported number of baboons were exposed to "heavy" concentrations of asbestos in food and drinking water for up to 5 yr. There was no evidence of any peritoneal or gastrointestinal tumors. The 5-yr exposure time appears too short for the carcinogenesis process to occur if the time element of the baboon's reaction to asbestos is similar to that of a human.

*Target Organ Toxicology Branch, Toxicology and Microbiology Division, Health Effects Research Laboratory, U.S. EPA, Cincinnati, OH 45268.

Table 1. Summary of asbestos ingestion studies.

Study	Species	Test material	Dose	Exposure time	Study duration	Number of animals, Initial/ Examined	Malignant tumors		
							Number	Location	Type[a]
Bonser (5)	Rat	Crocidolite	0.15% in diet *ad libitum*	To 78 wk	To 78 wk	40/12	0		
		Control	0	0	To 86 wk	65/25	1	Liver	S
Gross (7)	Rat	Chrysotile	5% in diet *ad libitum*	21 mo.	21 mo.	10/10	0		
		Control	0	0	21 mo.	5/5	0		
		Chrysotile	10 mg/wk	16 wk	To 1.5 yr	31/31	2	Breast	C
		Crocidolite	5 mg/wk	16 wk	To 1.5 yr	33/33	0		
		Crocidolite	10 mg/wk	16 wk	To 1.5 yr	34/34	1	Node	L
		Control	0	0	To 1.5 yr	24/24	5	3 Breast Thigh Node	C S L
		Crocidolite (2 sources)	10 mg/wk	18 wk	To 1.5 yr	63/63	0		
		Control	0	0	To 1.5 yr	24/24	0		
Gibel (8)	Rat	Chrysotile	20 mg/day	Life	441[b]	50/42	12	Lung 4 Kidney 3 Node 4 Liver	C C L C
		Talc	20 mg/day	Life	649[b]	50/45	3	Liver	C
		Control	0	0	702[b]	50/49	2	Liver	C
Cunningham (9)	Rat	Chrysotile	1% in diet *ad libitum*	To 24 mo.	To 24 mo.	10/7	6	Brain Pituitary Node 2 Kidney Peritoneum	S C L C S
		Control	0	0	To 24 mo.	10/8	1	Peritoneum	S
		Chrysotile	1% in diet *ad libitum*	To 24 mo.	To 30 mo.	40/36	11	2 Thyroid Thyroid Liver Chemodectoma jugular body Colon Ileum Adrenal 2 Node Bone	C S C C S C L S
		Control	0	0	To 30 mo.	40/32	11	Thyroid Liver 2 Adrenal Kidney Node 5 Fat	C C C C L S

Table 1. Summary of asbestos ingestion studies (continued).

Study	Species	Test material	Dose	Exposure time	Study duration	Number of animals, Initial/ Examined	Malignant tumors		
							Number	Location	Type[a]
Wagner (*10*)	Rat	Chrysotile	100 mg/day	101 days/ 5 mo.	619[b]	32/32	3	Node Stomach Uterus	L S S
		Talc	100 mg/day	101 days/ 5 mo.	614[b]	32/32	3	2 Uterus Stomach	S S
		Control	0	0	641[b]	16/16	0		
Smith (*11*)	Hamster	Amosite	0.5 mg/L *ad libitum*	To 23 mo.	To 23 mo.	60/60	1	Lung	C
		Amosite	5 mg/L *ad libitum*	To 23 mo.	To 23 mo.	60/60	3	2 Stomach Peritoneal mesothelioma	C
		Amosite	50 mg/L *ad libitum*	To 23 mo.	To 23 mo.	60/60	0		
		Taconite tailings	0.5 mg/L *ad libitum*	To 23 mo.	To 23 mo.	60/60	1	Uterus	S
		Taconite tailings	5 mg/L *ad libitum*	To 23 mo.	To 23 mo.	60/60	0		
		Taconite tailings	50 mg/L *ad libitum*	To 23 mo.	To 23 mo.	60/60	0		
		Control	0	0	To 23 mo.	120/120	1	Node	L
Donham (*12*)	Rat	Chrysotile	10% in diet *ad libitum*	To 32 mo.	To 32 mo.	240/189	4	3 Colon Abdominal mesothelioma	C
		Cellulose fiber	10% in diet *ad libitum*	To 32 mo.	To 32 mo.	242/197	2	Colon	C
		Control	0	0	To 32 mo.	121/115	3	Colon	C
Ward (*13*)	Rat	Azoxymethane[c]	7.4 mg/kg wk	10 wk	34 wk	21/21	12	5 Ileum 7 Colon	C C
		Azoxymethane plus amosite	7.4 mg/kg wk 10 mg 3/wk	10 wk	34 wk	21/18	10	3 Ileum 7 Colon	C C
		Azoxymethane plus chrysotile	7.4 mg/kg wk 10 mg 3/wk	10 wk	34 wk	21/21	10	4 Ileum 6 Colon	C C

Table 1. Summary of asbestos ingestion studies (continued).

Study	Species	Test material	Dose	Exposure time	Study duration	Number of animals, Initial/ Examined	Malignant tumors		
							Number	Location	Type[a]
Ward (*13*)	Rat	Amosite	10 mg 3/wk	10 wk	34 wk	21/21	0		
		Chrysotile	10 mg 3/wk	10 wk	34 wk	21/21	0		
		Saline	1.0 mL 3/wk (gavage)	10 wk	34 wk	21/21	0		
		Untreated	—	0	34 wk	21/21	0		
		Azoxymethane	7.4 mg/kg wk	10 wk	To 95 wk	50/48	39	12 Ileum 27 Colon	C C
		Azoxymethane plus amosite	7.4 mg/kg wk 10 mg 3/wk	10 wk	To 95 wk	50/48	44	15 Ileum 29 Colon	C C
		Saline plus amosite	1/wk (SC) 10 mg 3/wk	10 wk	To 95 wk	50/49	17	Ileum 16 Colon	C C
Hiding (*14*)	Rat	Filtered Duluth tapwater	1 mfl[d] *ad libitum*	690[b]	690[b]	28/27	3	Lung Ovary Forestomach	C C C
		Unfiltered Duluth tapwater	100 mfl *ad libitum*	960[b]	960[b]	30/28	4	Salivary gland Skin Uterus Mediastinum	C C S L
		Lake Superior water sediment	5,000 mfl *ad libitum*	840[b]	840[b]	22/22	3	Lung Skin Uterus	C C S
		Taconite tailings	100,000 mfl *ad libitum*	870[b]	870[b]	30/30	3	Neck Chest wall Mediastinum	S S L
		Chrysotile/ amosite	20 mg/day	870[b]	870[b]	30/30	6	Breast 2 fibrous histiocytoma Skin Mediastinum Pleural mesothelioma	C C L
		Amosite	300 mg/day	750[b]	750[b]	20/20	1	Leukemia	
		Diatomaceous earth	20 mg/day	840[b]	840[b]	30/30	5	Salivary gland 2 Uterus Skin Peritoneal mesothelioma	C S C

Table 1. Summary of asbestos ingestion studies (continued).

Study	Species	Test material	Dose	Exposure time	Study duration	Number of animals, Initial/ Examined	Malignant tumors		
							Number	Location	Type[a]
Bolton (*15*)	Rat	Amosite	250 mg/wk	25 mo.	Life	24/24	1	Stomach	S
		Crocidolite	250 mg/wk	25 mo.	Life	22/22	1	Adrenal	C
		Chrysotile	250 mg/wk	25 mo.	Life	22/22	5	Fat Pleural histiocytoma, 2 Adrenal Plasma cell tumor	S C
		Margarine control	0	0	Life	24/24	4	2 Adrenal Bladder Peritoneum	C C S
		Control	0	0	Life	23/23	2	Fat Lymphoma	S

[a]Type C = carcinoma; S = sarcoma; L = lymphoma.
[b]Mean survival time in days.
[c]Azoxymethane given subcutaneously; saline administered by oral gavage or subcutaneously.
[d]mfl = million amphibole fibers/L

The results of a series of feeding experiments with different sources of chrysotile and crocidolite were reported by Gross et al. (*7*). This paper incorporated data from unpublished results of various studies conducted by three laboratories. Animals fed asbestos by gavage in butter or margarine for up to 21 months failed to provide evidence of a carcinogenic effect. The experiments were flawed for the following reasons: the number of rats in the experimental groups was small, the doses of asbestos were limited, significant information on experimental protocol was missing, and systematic histologic examination was not performed on a significant number of rats.

A study by Gibel et al. (*8*) was undertaken to feed asbestos filter material to rats because of the concern of the possible adverse health effects of erosion of asbestos from the filters used to purify commercial beverages. The filter material was composed of sulfated cellulose, a condensation resin and chrysotile asbestos (53%). The authors did not provide any information regarding the size and shape of the asbestos fibers that were incorporated into the filter material. Although 12 malignant tumors were noted in the asbestos-exposed group of rats and the mean survival time was decreased in the asbestos-treated group, the authors stated that no conclusions could be made from their test results regarding the pathogenesis of the tumors caused by the oral intake of asbestos material. The relationship of this study to asbestos carcinogenicity was also confounded by the presence of several substances in the filter material, which were not clearly identified.

Cunningham and co-workers (*9*) conducted two limited feeding studies with male Wistar rats. Chrysotile asbestos (1% with 5% corn oil) was added to rat chow and fed to the animals for 24 months or 30 months. In the first study, 10 rats were exposed to asbestos. Six of the seven rats autopsied were found to have tumors, while only one malignancy was observed in the control animals. In the larger study of 80 animals, equal numbers of malignant tumors were noted in the exposed and the control groups. The authors stated that trace amounts of asbestos can penetrate the walls of the gastrointestinal tract, but evidence that asbestos causes cancer by the oral route of administration was inconclusive.

Wagner et al. (*10*) fed 32 Wistar rats 100 mg/day of chrysotile or talc in malted milk for 101 days over a 5-month period. A slight decrease in survival time was observed in the two experimental groups. One gastric leiomyosarcoma was detected in each exposure group. Interpretation of the results of this experiment is difficult because of the small number of animals included in the study.

A study in Smith's laboratory (*11*), which was the first study to utilize a large number of ani-

mals, was designed to more closely simulate an environmental exposure to asbestos. Hamsters were exposed to either amosite fibers or taconite tailings at three different dose levels in drinking water. The control animals received Lake Superior water that had been filtered by either a 0.45-μm or 0.1-μm filter. A small number of malignant tumors was detected in the exposed groups, but these tumors were not specifically attributed to asbestos because no cancers were detected in the high dose groups. The particle size of the different fiber types was well characterized by the investigators.

Another large lifetime animal study by Donham et al. (*12*) was initiated to induce and to characterize colon lesions in F344 rats by feeding them high levels of asbestos (10% of feed). Because of the high level of asbestos in the feed, a nonnutritive cellulose fiber control group was included. In this study only the colon and rectum were examined microscopically. Although differences in the number of colon tumors were not statistically significant between the asbestos-fed animals and control groups, the researchers presented the following observations, which suggest that ingested asbestos is not inert in the colon: evidence of increased probability of asbestos-fed rats to develop colon lesions generally, evidence for unique mesothelioma in rats fed asbestos, evidence for a colonic cell regulator defect, and evidence for asbestos fiber penetration of colon mucosa.

Two experiments were designed by Ward et al. (*13*) to determine the promoter potential of oral exposure to asbestos. Could asbestos modify the response to azoxymethane, a known intestinal carcinogen? Rats were exposed to azoxymethane and/or asbestos for 10 weeks and were sacrificed 34 weeks later or observed throughout their lifespan. In the first experiment, intestinal carcinomas were detected only in the groups of animals receiving azoxymethane alone or in combination with asbestos. In the second experiment of longer duration, the incidence of intestinal tumors was only slightly greater in the amosite plus azoxymethane group as compared with the azoxymethane group. Furthermore, the authors concluded that amosite alone caused a relatively high rate of intestinal neoplasia. However, there were no control animals included in the second experiment, which compromises the findings. The researchers reported a 14% incidence of Zymbal gland tumors in the rats exposed only to amosite. The historical rate of Zymbal gland tumors in the National Cancer Institute Bioassay Program is 0.34%, indicating that it is a relatively rare tumor. Since a single dose of azoxymethane has been shown to induce both Zymbal gland tumors and intestinal carcinomas (*14*), an inadvertent exposure to azoxymethane might have caused the high incidence of intestinal neoplasma and Zymbal gland tumors in the amosite-exposed animals. One has reason to doubt the authors' conclusion that oral asbestos exposure in F344 rats may have increased the incidence of intestinal tumors occurring naturally.

Since amphibole fibers had been detected in Lake Superior and in the Duluth municipal water supply, a study was conducted by Hilding et al. (*15*) to investigate the potential carcinogenic effect of unfiltered Duluth tapwater, municipal water reservoir sediments, taconite plant tailings, amosite, and diatomaceous earth. Under the experimental conditions of this study, no significant increases were detected in the incidence of malignant tumors in any experimental group when compared to controls.

The final study (*16*) considered in this review examined the effects of prolonged asbestos exposure to rats. Animals were fed over 250 mg/week of amosite, crocidolite and chrysotile in margarine for periods up to 25 months. No excess of malignant tumors were found in any experimental group, and no gastrointestinal mucosal abnormalities were detected. Bolton and co-workers concluded that there were no significant adverse health effects from prolonged asbestos ingestion in healthy laboratory rats.

Conclusions

Certain conclusions can be summarized from the various ingestion studies. The bulk of the experimental evidence indicates that the long-term, high-level ingestion exposure to various types of asbestos fibers failed to produce any definite, reproducible, organ-specific carcinogenic effect. Although comparisons between studies are confounded by different rat strains utilized, by different dose levels or exposure conditions, and by different types of asbestos employed, the vast majority of the asbestos ingestion studies were either negative or equivocal. There was apparently a carcinogenic response to amosite in one study (*13*), but the authors did not rule out that an inadvertent exposure to azoxymethane, an intestinal carcinogen, had occurred. Many of the studies suffered from an insufficient number of experimental animals and from an inadequate exposure time to asbestos. Another major drawback of many of the studies was that they were not lifetime studies.

One can question the suitability of the animal models employed in evaluating the human response to oral exposure to asbestos, since sufficient time may be lacking between exposure and the development of malignancies during the animal's lifetime. However, exposure to asbestos by other routes has induced cancer in rats. For example, Wagner et al. (*17*) reported the development of lung cancer and mesothelioma from brief to lengthy inhalation exposure to various types of asbestos, while Gross (*18*) reported asbestotic lung cancers in 25 of 72 rats that survived 16 months of exposure to chrysotile dust. Based on the carcinogenic effects of asbestos from nonoral exposure routes (*2,3*), one would expect to be able to produce a neoplastic response within the lifetime of conventional laboratory animals with massive doses of ingested asbestos such as those employed in some of the studies mentioned in this paper. These studies also cast some doubt on the hypothesis that peritoneal mesotheliomas and gastrointestinal cancers result from the ingestion of asbestos fibers cleared from the lungs following inhalation exposure.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement of recommendation for use.

REFERENCES

1. Bogovski, P., Timbrell, V., Gilson, J. C., and Wagner, J. C. (Eds.). Biological Effects of Asbestos. IARC Scientific Publications, Lyon, 1973.
2. Selikoff, I. J., and Lee, D. H. K. Asbestos and Disease. Academic Press, New York, 1978.
3. Peters, G. A., and Peters, B. J. Sourcebook on Asbestos Diseases. Garland STPM Press, New York, 1980.
4. Cook, P. M., Glass, G. E., and Tucker, J. H. Asbestiform amphibole minerals: detection and measurement of high concentrations in municipal water supplies. Science 185: 853–855 (1974).
5. Bonser, G. M., and Clayson, D. B. Feeding of blue asbestos to rats. Brit. Emp. Cancer Campaign, Res. Ann. Rept. 45: 242 (1967).
6. Webster, I. The ingestion of asbestos fibers. Environ. Health Perspect. 9: 199–202 (1974).
7. Gross, P., Harley, R. A., Swinburne, L. M., Davis, J. M. G., and Greene, W. B. Ingested mineral fibers. Arch. Environ. Health 29: 341–347 (1974).
8. Gibel, W., Lohs, K., Horn, K. H., Wildner, G. P., and Hoffman, F. Tierexperimentelle Untersuchungen uber eine Kanserogene Wirkung von Asbesfiltermaterial nach oraler Aufnahme (Experimental study on carcinogenic activity of asbestos filters following oral ingestion). Arch. Geschwulstforsch. 46: (6): 437–442 (1976).
9. Cunningham, H. M., Moodie, C. A., Lawrence. G. A., and Pontefract, R. D. Chronic effects of ingested asbestos in rats. Arch. Environ. Contam. Toxicol. 6: 507–513 (1977).
10. Wagner, J. C., Berry, C., Cook, T. J., Hill, R. J., Pooley, F. D., and Skidmore, J. W. Animal experiments with talc. In: Inhaled Particles IV (W. H. Walton, Ed.), Pergamon Press, New York, 1977, pp. 647–654.
11. Smith, W. E., Hubert, D. D., Sobel, H. J., Peters, E. T., and Doerfler, T. E. Health of experimental animals drinking water with and without amosite asbestos and other mineral particles. J. Environ. Pathol. Toxicol. 3: 277–300 (1980).
12. Donham, K. J., Berg, J. W., Will, L. A., and Leininger, J. R. The effects of long-term ingestion of asbestos on the colon of F344 rats. Cancer 45: 1073–1084 (1980).
13. Ward, J. M., Frank, A. L., Wenk, M., Devor, D., and Tarone, R. E. Ingested asbestos and intestinal carcinogenesis in F344 rats. J. Environ. Pathol. Toxicol. 3: 301–312 (1980).
14. Ward, J. M. Dose response to a single injection of azoxymethane in rats. Vet. Pathol. 12: 165–177 (1975).
15. Hilding, A. C., Hilding, D. A., and Larson, D. M. Biological effects of ingested amosite asbestos taconite tailings, diatomaceous earth, and Lake Superior water in rats. Arch. Environ. Health 36: 298–303 (1981).
16. Bolton, R. E., Davis, J. M. G., and Lamb, D. The pathological effects of prolonged asbestos ingestion in rats. Environ. Res. 29: 134–150 (1982).
17. Wagner, J. C., Berry, G., Skidmore, J. W., and Timbrell, V. The effects of the inhalation of asbestos in rats. Brit. J. Cancer 29: 252–269 (1974).
18. Gross, P., deTreville, R. T. P., Tolker, E. B., Kaschak, M., and Babyak, M. A. Experimental asbestosis: the development of lung cancer in rats with pulmonary deposits of chrysotile asbestos dust. Arch. Environ. Health 15: 343–355 (1967).

Environmental Health Perspectives
Vol. 53, pp. 11–25, 1983

Chronic Effects of Dietary Exposure to Amosite and Chrysotile Asbestos in Syrian Golden Hamsters

by Ernest E. McConnell,* Alan M. Shefner,† John H. Rust† and John A. Moore*

Bioassays of amosite, short-range (SR), intermediate-range (IR) or intermediate-range chrysotile asbestos in combination with the intestinal carcinogen 1,2-dimethylhydrazine dihydrochloride (DMH) were conducted with male and female Syrian golden hamsters. Amosite and both forms of chrysotile asbestos were administered at a concentration of 1% in pelleted diet for the entire lifetime of the hamsters starting with mothers of the test animals. Group sizes varied from 125-254. There was no adverse effect on body weight gain or survival by either type of asbestos or by IR chrysotile asbestos in combination with DMH.

A significant increase ($p < 0.05$) in adrenal cortical tumors was observed in male hamsters exposed to SR and IR chrysotile asbestos and in females treated with IR chrysotile asbestos when compared to the pooled control groups. However, statistical significance ($p < 0.05$) was lost when these dosed groups were compared with temporal control groups. Neither of the male or female amosite asbestos groups showed increased neoplasia in any tissue or organ compared to the control groups. The cocarcinogen studies using IR chrysotile asbestos and 1,2-dimethylhydrazine dihydrochloride were considered inadequate because there was no increase in intestinal neoplasia in the DMH group.

Introduction

In November 1973 the National Institute of Environmental Health Sciences and the Environmental Protection Agency cosponsored a symposium on the possible biological effects of ingested asbestos (*1*). This conference concluded that a paucity of definitive data existed concerning the effects of ingested asbestos and that specific research was needed.

A subcommittee of the DHEW Committee to Coordinate Toxicology and Related Programs was established to review existing data and to prepare a draft research protocol that would be responsive to the possible public health implication of ingested asbestos. This protocol was widely distributed for comment within and outside the government and a public meeting of the subcommittee was held on February 11, 1975. On the basis of the comments received, a revised final protocol was developed which called for the use of long-term animal toxicology studies to evaluate the ingestion of several minerals for carcinogenic effect. As a result the National Toxicology Program investigated the carcinogenic potential of the ingestion of various forms of asbestos in rats and hamsters. All of the studies were to encompass the lifetime of the animal, including exposure of the dams from which the test animals were derived.

This report represents the results of studies undertaken to determine the effects of the ingestion of amosite and chrysotile (short- and intermediate-range) asbestos in Syrian golden hamsters. The effects of intermediate-range chrysotile asbestos in conjunction with a known intestinal carcinogen, 1,2-dimethylhydrazine (DMH), were also studied.

*National Toxicology Program, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 22709.

†IIT Research Institute, Life Sciences Research Division, 10 West 35th Street, Chicago, IL 60616.

Table 1. Fiber characteristics of chrysotile asbestos.

Fiber characteristics	Short-range	Intermediate-range
Surface area, m^2/g	59.0	27.9
Density, g/cm^3	2.577 ± 0.022 SD	2.607 ± 0.016 SD
Measurement, transmission electron microscopy		
Fiber count/g	0.6081×10^{13}	0.1291×10^{12}
Median length, μm	0.66	0.82
Range of length, μm	0.088–51.1	0.104–783
Median diameter, μm	0.059	0.089
Range of diameter, μm	0.019–1.57	0.019–11.5
Median fiber aspect ratio (L/D)	11.1698	8.435
Frequency distribution by length (μm), optical microscopy		
10 percentile	1.3	1.4
20 percentile	1.7	1.9
30 percentile	2.2	3.0
40 percentile	2.6	5.4
50 percentile	3.1	14.0
60 percentile	3.8	29.0
70 percentile	4.5	48.0
80 percentile	5.8	76.0
90 percentile	7.8	130.0

Materials and Methods

Asbestos is a general term applied to certain natural mineral silicates when they appear in a fibrous form. Chrysotile is the fibrous member of the serpentine mineral group while amosite is an amphibole mineral. Two chrysotile test materials were selected for testing and are referred to as short-range (SR) and intermediate-range (IR) chrysotile. Intermediate-range chrysotile differs from short-range chrysotile in that the former contains fibers extending into relatively large sizes, both with respect to length and diameter.

The short-range chrysotile was purchased from the Union Carbide Corporation, Niagara Falls, New York, which referred to the material as COF-25. The chrysotile was mined from the New Idria serpentine mass located in the southwestern San Benito and western Fresno counties of California. Mineral and fiber characteristics of SR chrysotile are shown in Tables 1 and 2.

The intermediate-range chrysotile was purchased from the Johns Manville Company, which referred to the material as Plastobest-20. The chrysotile was obtained from the Jeffrey Mine, Asbestos, Quebec, Canada. Mineral and fiber characteristics of IR chrysotile are shown in Tables 1 and 2.

An amosite sample (S-33) from a mine in Penge, Transvaal, Republic of South Africa was purchased by the Bureau of Mines from the Atlas Asbestos Company, Montreal, Quebec, Canada. The sample was processed by a single pass through an air jet mill to improve the homogeneity of the amosite. Mineral and fiber characteristics of amosite are shown in Tables 3-5.

Table 2. Chemical-instrumental analyses of chrysotile asbestos.

	Content, wt—%	
	Short-range	Intermediate-range
Al_2O_3	0.66	1.47
CaO	0.32	0.05
Fe_2O_3	2.02	2.93
MgO	40.62	40.62
K_2O	Not detected	0.08
SiO_2	39.77	39.90
Na_2O	0.01	0.04
TiO_2	0.03	0.04
MnO	0.07	0.06
Cr_2O_3	0.17	0.06
NiO	0.17	0.06
Co_2O_3	0.01	Not detected
CO_2	0.78	0.51
H_2O	1.54	1.17
H_2O^+	12.69	12.81
Benzene-extracted organics	0.026	0.011

The homogenicity of the samples and the physical and chemical properties of the materials were extensively characterized by the Bureau of Mines, U.S. Department of the Interior (Supt. of Documents No. I 28.23:8452) and by the Fine Particle Laboratories, Illinois Institute of Technology Research Institute, Chicago, Illinois (Special Report and Addendum on project L6085, contract No1-ES-5-3157). Copies of these reports are available upon request from the National Toxicology Program.

Test Diets

The feed used was NIH-31 open formula rodent diet prepared by Zeigler Brothers, Inc., Gardner,

PA. The appropriate asbestos was incorporated to a level of 1% by weight into the test diet. All feed was pelleted with a Sprout-Waldron pelleter; the pellets were of oval configuration, 3/8 in. by 3/4 in. in size. Pelleted feed was packaged in 25-lb aliquots in standard paper feed-bags which were color coded to minimize the occurrence of feeding errors at the test laboratory. Each lot of blended feed was analyzed for asbestos concentration.

Table 3. Fiber characteristics of amosite asbestos.

Fiber characteristics	
Surface area, m^2/g	4.13
Density, g/cm^3	3.35 ± 0.026 SD
Measurements, transmission electron microscopy	
Fiber count/g	0.3466×10^{10}
Median length, μm	4.37
Range of length, μm	0.85–995
Median diameter, μm	0.72
Range of diameter, μm	0.064–12.4
Median fiber aspect ratio (*L/D*)	6.4248

Table 4. Chemical-instrumental asbestos of amosite asbestos.

Al_2O_3	0.42
CaO	0.48
FeO	34.61
Fe_2O_3	2.24
MgO	6.22
K_2O	0.30
SiO_2	50.36
Na_2O	0.03
MnO	2.66
Cr_2O_3	0.03
NiO	0.01
CO_2	0.88
H_2O	0.15

Sources and Specifications of Test Animals

Four groups (three chrysotile and one amosite) of disease-free, mated female outbred Syrian golden hamsters were obtained over a period of 20 weeks in 1977 from Charles River Lakeview Laboratories, New Field, NJ. The hamsters had been mated 6 days prior to shipping.

Animal Maintenance

Upon arrival, the mated female hamsters were weighed and sorted into weight ranges. They were then distributed randomly between control and treatment groups, which were housed in separate rooms. The first shipment of mated females was assigned to the short-range (SR) chrysotile study, the second to the intermediate-range (IR) chrysotile study, the third shipment to the IR chrysotile plus DMH study, the fourth group to the amosite study and their respective control groups. Each dam was placed in an individual cage with filter top in its respective room. Control or formulated diets were provided *ad libitum* on the floor of each cage. Water was provided *ad libitum* via bottles. The hamsters were not handled just before the litters were due to be born except when the cages were changed. Once the litters were born, they were left undisturbed until they were approximately 10 days of age. Then, the cages were changed weekly until the offspring

Table 5. Particle size distribution of amosite asbestos by particle number: SEM.[a]

	Length interval, μm						
	0–1.99	2–3.99	4–5.99	6–7.99	8–9.99	10–19.99	20–39.99
Amosite mean width, μm.	0.28	0.38	0.45	0.45	0.48	0.52	0.51
Amosite particles per interval	57	126	83	78	52	181	184
Total amosite particles, %	5.6	12.3	8.6	7.6	5.1	17.7	18.0
Cumulative amosite, %	5.6	17.9	26.5	34.1	39.2	56.9	74.9
Amosite, vol.-%	–	0.1	0.3	0.4	0.4	2.4	5.0
Cumulative vol-% amosite	–	0.1	0.4	0.8	1.2	3.6	8.6
Number of other particles	11	8	1	0	1	1	0
Amosite particles per length interval, % by aspect ratio:							
1:1–2.9:1	12	0	0	0	0	0	0
3:1–4.9:1	34	10	6	5	2	0	0
5:1–9.9:1	43	52	23	14	4	1	1
10:1–19.9:1	11	34	52	38	40	21	1
20:1–49.9:1	0	4	18	41	54	64	30
50:1–99.9:1	0	0	1	2	0	12	55
100:1–199:1	0	0	0	0	0	2	12
200:1–499:1	0	4	0	0	0	0	1
>500:1	0	0	0	0	0	0	0

[a]Calculated from particle number data, assuming rectangular cross section with third dimension equal to 1/2 measured width.

were 4 weeks of age, at which time they were weaned.

At weaning, the offspring were individually weighed and separated by sex. The test groups were randomly placed into groups of three males and three females and housed in polycarbonate cages for the remainder of the lifetime study. The dams were killed at this time. Twenty male and twenty female offspring were removed from the study for endo- and ectoparasite examination to confirm that the test groups were of a desired health status. Extra hamsters were not discarded at this time, in case animals had been missexed. Approximately 6 weeks after weaning, all missexed hamsters were killed along with their cage mates and were replaced with these alternates which had received maintenance identical to that received by the original hamsters. The remaining hamsters were killed. The experimental design insured that ingestion of asbestos spanned the entire phase of solid food consumption during the lifetime of the animal. Food consumption was not determined because of the hamster's habit of sequestering its feed in the bedding. Control hamsters were housed in separate rooms. The number of animals in the study is shown in Table 6.

Starting at 6 weeks of age, male and female hamsters in the intermediate-range chrysotile/1,2-dimethylhydrazine dihydrochloride (DMH) study (Table 1) were given oral doses of DMH (4 mg/kg) every other week for a total of 5 doses. The dose of DMH used in this study was based on the results of a pilot study carried out previously in the same facility. The latter was conducted in a manner similar to that reported in rats (2). The DMH (Aldrich Chemical Co., Milwaukee, WI) was used as received and was dissolved in 0.9% saline to a concentration of 1.5% (15 mg/mL). This stock solution was then diluted with saline to give the appropriate concentration for dosing. The solutions were made up within one hour prior to the dosing of the hamsters. All dosing was completed in less than 3 hr. The DMH was analyzed after each dosing.

During the test period, room temperature was maintained at 22 ± 2°C and the relative humidity ranged from 40% to 80%. To minimize contamination of room air with asbestos, each cage was totally enclosed. Incoming air was filtered to the cages through glass fiber filters while exit air was filtered through a fiberglass roughing filter followed by a bag housing filter. The cage atmosphere was negative relative to the room and the room was maintained at a slightly negative atmosphere in relation to corridor air. Air flow within the animal rooms was maintained with a minimum of 20 air changes/hr. Flourescent lighting was provided 12 hr/day.

Clinical Examinations and Pathology

All hamsters were observed daily for signs of toxicity. Body weights of individual animals were recorded weekly for the duration of the study. All animals were allowed to die or were killed with pentobarbital sodium when moribund. A complete postmortem examination was performed on all animals not severely cannibalized or autolyzed. Thus, the number of animals from which particular organs or tissues were examined mi-

Table 6. Disposition of hamsters in oral asbestos study.

Group	Sex	On test	Histopathologic evaluation	Missing	Cannibalized	Autolyzed	Missexed
SR chrysotile	M	126	115	0	3	6	2
control	F	126	114	0	1	6	5
IR chrysotile	M	126	116	0	0	8	2
control	F	126	119	0	0	4	3
DMH and IR chrysotile	M	125	119	0	0	3	3
control	F	128	120	1	0	2	5
Amosite	M	127	122	0	0	4	1
control	F	126	119	1	0	1	5
SR chrysotile	M	253	233	0	1	10	9
	F	252	228	1	0	17	6
IR chrysotile	M	251	245	0	0	3	3
	F	252	244	1	0	3	4
DMH	M	127	127	0	0	0	0
	F	126	122	0	0	1	3
DMH and IR	M	176	173	0	0	2	1
chrysotile	F	174	161	3	0	6	4
Amosite	M	252	248	0	0	3	1
	F	254	237	5	0	5	7

croscopically varies and does not necessarily represent the number of animals that were placed on study in each group (Table 6).

Since the gastrointestinal tract was chosen as one of the target organs prior to the study, it was handled in a manner slightly different from the usual in standard rodent lifetime bioassays. Prior to placement in fixative, the entire esophagus was opened and pinned with the exterior surface adjacent to cardboard. The stomach and cecum were prepared similarly. Lengths of duodenum and ileum (2 cm) and two portions of jejunum were placed unopened in fixative. The remaining small intestine was opened and gently washed with saline and it was then carefully examined. Suspect lesions were processed separately and identified individually as to location. Likewise, the entire colon with anus was opened, examined, and pinned to cardboard prior to fixation. The size and location of masses were recorded. Masses greater than 1 mm in diameter were removed as separate specimens for processing. After fixation and prior to embedding, the colon was "carpet-rolled" starting at the posterior end, with the mucosal surface inward.

All tissues were fixed in 10% neutral buffered formalin, sectioned, and stained with hematoxylin and eosin. Tissues/organs examined microscopically were: tissue masses, the above mentioned portions of gastrointestinal tract, mesenteric and bronchial lymph nodes, mammary gland, salivary gland, thigh muscle, bone marrow (sternum), nasal cavity with turbinates, larynx, trachea, lungs, and bronchi, heart, thyroid, parathyroid, liver, gallbladder, pancreas, spleen, kidneys, adrenal glands, urinary bladder, seminal vesicles/prostate/testes, ovaries/uterus, brain, pituitary gland, eyes and spinal cord. Selected sections were stained with Bennhold's congo red to demonstrate amyloid.

Data Recording and Statistical Methods

The individual animal pathology data on this experiment were recorded in the Carcinogenesis Bioassay Data System. The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, and individual pathologic results.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (*3*). Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Differences in survival were evaluated by Cox's life table method (*4*).

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

For the statistical analysis of tumor incidence data, two different methods of adjusting for intercurrent mortality were employed. Each used the classical methods for combining contingency tables developed by Mantel and Haenszel (*5*).

The first method of analysis assumed that all tumors of a given type were fatal, i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the treated and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results were then combined by the Mantel-Haenszel methods to obtain an overall probability (p) value. This method of adjusting for intercurrent mortality is Cox's life table method (*4*).

The second method of analysis assumed that all tumors of a given type were "incidental," i.e., they were merely observed at autopsy in animals dying of an unrelated cause. According to this approach, the proportions of animals found to have tumors in treated and control groups were compared in each of five time intervals. For male hamsters these time intervals were 0–52 weeks, 53–78 weeks, 79–92 weeks, 93–103 weeks and beyond 103 weeks. For female hamsters whose median survival was considerably less than that of the males, the time intervals were 0–44 weeks, 45–52 weeks, 53–60 weeks, 61–68 weeks and beyond 68 weeks. The denominators of these proportions were the number of animals actually autopsied during the time interval. The individual time interval comparisons were then combined by the previously described methods to obtain a single overall result (*6*).

In addition to these tests, one other set of statistical analyses were carried for each primary tumor: the Fisher exact test based on the overall

proportion of tumor-bearing animals(7). All reported p values are one-sided. Except where noted, the three alternative analyses gave similar results.

Results

Establishment of Test Groups

The experiment was designed to evaluate the effects of orally ingested amosite or chrysotile asbestos during the entire life of the animal, starting from the time it was able to eat solid food. For this reason, the mated female hamsters had been on the test diets for approximately 2 weeks when the first litters were born. Of the females, 10–15% were not pregnant, aborted, or their litters died immediately after birth. Several more dams died after showing a prolapsed rectum in the week following birth. The incidences of infertility and neonatal deaths were unrelated to the test diet. To minimize the chance that the mothers would reject or cannibalize their young, the litters were not handled during lactation. Many of the pups which died during the nursing period were cannibalized by their mothers. In those pups in which a postmortem examination was possible, the stomachs were typically without food (milk), suggesting maternal rejection or inability to compete with litter mates. None of these observations were compound-related.

Approximately 2% of the offspring in all groups died between weaning and 14 weeks of age due to cage fighting or an enteritis of undetermined origin. Histologically, the disease was compatible with the acute form of proliferate ileitis ("wet tail"), a common disease of hamsters. Combinations of cage fighting and enteritis were also observed. These deaths were not compound-related, although cage fighting was more severe in the SR chrysotile and its temporal control group than in the other two portions of the study. Replacement hamsters were incorporated into the groups (in additional cages) to maintain group sizes until the animals were 12 weeks of age; from this time on, no additional hamsters were added to the experimental groups. The extra hamsters were killed.

Body Weights and Clinical Signs

Body weight gain was not adversely affected in any dose group, including the group given 1,2-dimethylhydrazine dihydrochloride (DMH). In fact, both types of asbestos appeared to increase body weight in most of the dosed groups.

No compound-related clinical signs were observed during the entire study. Occasional skin lesions and bite wounds were observed in both sexes, but were more apparent in males; these became less of a problem after the hamsters were 20 weeks of age.

Survival

Survival was not adversely affected by any of the test diets with the possible exception of DMH-treated female hamsters. Survival rates were actually higher in the amosite and SR and IR chrysotile groups relative to the temporal controls. The median life-span of females (control and treated) was shorter than that of corresponding groups of males (Table 7). The median survival of control

Table 7. Median life span of hamsters in oral asbestos study.

Group	Sex	Median life span, weeks
IR chrysotile control	M	83
	F	61
IR chrysotile	M	86
	F	59
SR chrysotile control	M	77
	F	57
SR chrysotile	M	87
	F	63[a]
DMH and IR chrysotile control	M	82
	F	57
DMH	M	82
	F	54[b]
IR chrysotile and DMH	M	90
	F	63[a]
Amosite control	M	81
	F	55
Amosite	M	84
	F	60

[a]Significantly ($p < 0.05$) improved overall survival relative to controls (life table analysis).
[b]Significantly ($p < 0.05$) reduced overall survival relative to controls (life table analysis).

female groups was 55–61 weeks, compared to 77–83 weeks for control male hamsters (Table 7).

Pathology

The number of hamsters available for histopathologic examination is shown in Table 6. Most animals not examined pathologically were excluded because of autolysis or cannibalization. Review of the clinical records of hamsters lost to autolysis or cannibalization gave no indication that these animals had neoplasia.

A variety of neoplasms was observed in control (Tables 8 and 9) and asbestos-exposed hamsters (Tables 10–17). The proportion of control male or female hamsters bearing primary tumors was not statistically different among the four control groups. Thus, statistical comparisons were made with pooled controls as well as with temporal controls. Overall, the male hamsters had a slightly higher rate of neoplasia than the females.

Those organs with greater than 4% incidence of neoplasia in dosed or control groups were the adrenal gland, pancreas (islets of Langerhans), parathyroid, and reticuloendothelial system.

The only organ showing an increased rate of neoplasia in chrysotile-exposed hamsters compared to the controls was the adrenal cortex. In male hamsters, the incidence of cortical adenomas was significantly increased ($p < 0.01$) in the SR and IR chrysotile groups compared with the pooled controls but not in the DMH chrysotile or amosite groups. None of the chrysotile groups showed a significant ($p < 0.05$) increase in cortical adenomas relative to their temporal control groups. A similar increase in cortical adenomas was observed in the female IR chrysotile group compared with pooled controls, but this also ceased to be significant when compared with the temporal control group.

In only three other instances did specific tumor types show significant effects relative to pooled or temporal controls. The only statistically significant ($p < 0.05$) difference in tumor incidence observed in the amosite study was a decrease in islet-cell adenomas observed in female hamsters. Female hamsters administered SR chrysotile showed a significantly ($p < 0.05$) decreased incidence of islet-cell adenoma relative to pooled controls (Table 16). Male hamsters administered DMH showed a significantly ($p < 0.05$) increased incidence of leukemia or malignant lymphoma relative to pooled controls (Table 16).

The only group to show a significant ($p < 0.05$) increase in overall primary tumors was the male IR chrysotile group. This increase was due primarily to adrenal tumors. Male hamsters receiving SR chrysotile or DMH and IR chrysotile also

Table 8. Incidence of primary tumors in male hamster control groups.

	Short-range chrysotile control	Intermediate-range chrysotile control	DMH and intermediate-range chrysotile control	Amosite control
Animals with primary tumors	21/115 (18%)	26/116 (22%)	27/119 (23%)	21/122 (17%)
Skin or subcutaneous tissue, all tumors	0/115 (0%)	1/116 (1%)	1/119 (1%)	0/122 (0%)
Lung or trachea, all tumors	0/115 (0%)	0/116 (0%)	9/119 (0%)	0/120 (0%)
Adrenal				
Cortical adenoma	7/115 (6%)	7/115 (6%)	3/117 (3%)	8/119 (7%)
Cortical carcinoma	3/115 (3%)	3/115 (3%)	4/117 (3%)	3/119 (3%)
Pheochromocytoma	2/115 (2%)	5/115 (4%)	3/117 (3%)	3/119 (3%)
Other tumors	0/115 (0%)	3/115 (3%)	2/117 (2%)	1/119 (1%)
Pancreas				
Islet-cell adenoma	2/111 (2%)	7/110 (6%)	8/110 (7%)	3/114 (3%)
Islet-cell carcinoma	1/111 (1%)	0/110 (0%)	0/110 (0%)	0/114 (0%)
Thyroid				
C-cell adenoma	3/109 (3%)	3/106 (3%)	0/107 (0%)	1/106 (1%)
C-cell carcinoma	1/109 (1%)	1/106 (1%)	0/107 (0%)	1/106 (1%)
Other tumors	0/109 (0%)	0/106 (0%)	1/107 (1%)	0/106 (0%)
Parathyroid, adenoma	0/72 (0%)	1/71 (1%)	1/64 (2%)	0/64 (0%)
G.I. tract, all tumors	2/115 (2%)	1/116 (1%)	2/119 (2%)	1/112 (1%)
Pituitary, all tumors	0/84 (0%)	0/77 (0%)	0/80 (0%)	0/81 (0%)
Kidney, all tumors	0/115 (0%)	2/116 (2%)	1/119 (1%)	1/120 (1%)
Liver, all tumors	0/115 (0%)	0/116 (0%)	0/119 (0%)	0/120 (0%)
Leukemia or malignant lymphoma	2/115 (2%)	1/116 (1%)	4/119 (3%)	1/122 (1%)
Hemangioma or hemangiosarcoma	0/115 (0%)	0/116 (0%)	3/119 (3%)	2/122 (2%)
All other tumors	1/115 (1%)	0/116 (0%)	3/119 (3%)	1/122 (1%)

showed an elevated incidence of primary tumors relative to pooled controls. However, when survival differences were taken into account by a life table analysis, these differences were not statistically significant. Female chrysotile groups showed little evidence of an increased incidence of primary tumors relative to temporal or pooled controls.

Table 9. Incidence of primary tumors in female hamster control groups.

	Short-range chrysotile control	Intermediate-range chrysotile control	DMH and intermediate-range chrysotile control	Amosite control
Animals with primary tumors	19/114 (17%)	17/119 (14%)	15/120 (12%)	15/119 (13%)
Skin or subcutaneous tissue, all tumors	0/114 (0%)	1/119 (0%)	1/120 (0%)	0/119 (0%)
Lung or trachea, all tumors	0/114 (0%)	0/119 (0%)	0/119 (0%)	0/119 (0%)
Adrenal				
Cortical adenoma	4/112 (4%)	6/118 (5%)	3/120 (2%)	2/118 (2%)
Cortical carcinoma	0/112 (0%)	0/118 (0%)	0/120 (0%)	0/118 (0%)
Pheochromocytoma	0/112 (0%)	0/118 (0%)	0/120 (0%)	0/118 (0%)
Other tumors	0/112 (0%)	0/118 (0%)	0/120 (0%)	0/118 (0%)
Pancreas				
Islet-cell adenoma	2/109 (2%)	5/116 (4%)	5/116 (4%)	3/115 (3%)
Islet-cell carcinoma	1/109 (1%)	0/116 (0%)	0/116 (0%)	0/115 (0%)
Thyroid				
C-cell adenoma	2/106 (2%)	3/115 (3%)	0/112 (0%)	1/106 (1%)
C-cell carcinoma	1/107 (0%)	1/115 (0%)	0/112 (1%)	1/106 (0%)
Other tumors	2/107 (2%)	0/115 (0%)	1/112 (0%)	0/106 (0%)
Parathyroid, adenoma	3/68 (4%)	1/77 (1%)	1/74 (1%)	1/61 (1%)
G.I. tract, all tumors	1/114 (1%)	2/119 (2%)	1/120 (1%)	1/119 (1%)
Pituitary, all tumors	0/77 (0%)	2/67 (3%)	0/62 (0%)	0/79 (0%)
Kidney, all tumors	0/114 (0%)	1/119 (1%)	0/120 (0%)	0/119 (0%)
Liver, all tumors	0/114 (0%)	0/119 (0%)	0/119 (0%)	0/118 (0%)
Leukemia or malignant lymphoma	2/114 (2%)	1/119 (0%)	3/120 (2%)	2/119 (2%)
Hemangioma or hemangiosarcoma	0/114 (0%)	0/119 (0%)	1/120 (1%)	1/119 (1%)
Uterus, all tumors	3/113 (3%)	1/119 (1%)	2/120 (2%)	2/119 (2%)
All other tumors	3/114 (3%)	0/119 (0%)	1/120 (1%)	2/119 (2%)

Table 10. Incidence of primary tumors in male hamsters administered amosite asbestos.

	Pooled controls	Amosite controls	Amosite-treated
Animals with primary tumors	95/472 (20%)	21/122 (17%)	57/248 (23%)
Skin or subcutaneous tissues, all tumors	2/472 (<1%)	0/122 (0%)	0/248 (0%)
Lung and trachea, all tumors	0/470 (0%)	0/120 (0%)	0/248 (0%)
Adrenal			
Cortical adenoma	25/466 (5%)	8/119 (7%)	13/246 (5%)
Cortical carcinoma	13/466 (3%)	3/119 (3%)	7/246 (3%)
Pheochromocytoma	13/466 (3%)	3/119 (3%)	4/246 (2%)
Other tumors	6/466 (1%)	1/119 (1%)	2/246 (1%)
Pancreas			
Islet-cell adenoma	20/455 (4%)	3/114 (3%)	11/234 (5%)
Islet-cell carcinoma	1/445 (<1%)	0/114 (0%)	0/234 (0%)
Thyroid			
C-cell adenoma	7/428 (2%)	1/106 (1%)	7/221 (3%)
C-cell carcinoma	1/428 (<1%)	0/106 (0%)	2/221 (1%)
Other tumors	1/428 (<1%)	0/106 (0%)	2/221 (1%)
Parathyroid, ademona	2/271 (1%)	0/64 (0%)	2/150 (1%)
G.I. tract, all tumors	6/472 (1%)	1/122 (1%)	6/248 (2%)
Pituitary, all tumors	0/322 (0%)	0/81 (0%)	0/182 (0%)
Kidney, all tumors	4/470 (1%)	1/120 (1%)	2/248 (1%)
Liver, all tumors	0/470 (0%)	0/120 (0%)	0/247 (0%)
Leukemia or malignant lymphoma	8/472 (2%)	1/122 (1%)	5/248 (2%)
Hemangioma or hemangiosarcoma	5/472 (1%)	2/122 (2%)	2/248 (1%)
All other tumors	5/472 (1%)	1/122 (1%)	2/248 (1%)

Table 11. Incidence of primary tumors in female hamsters administered amosite asbestos.

	Pooled controls	Amosite controls	Amosite-treated
Animals with primary tumors	66/472 (14%)	15/119 (13%)	30/237 (13%)
Skin or subcutaneous tissues, all tumors	0/472 (0%)	0/119 (0%)	2/237 (1%)
Lung and trachea, all tumors	0/471 (0%)	0/119 (0%)	0/234 (0%)
Adrenal			
Cortical adenoma	15/468 (3%)	2/118 (2%)	6/234 (3%)
Cortical carcinoma	0/468 (0%)	0/118 (0%)	0/234 (0%)
Pheochromocytoma	0/468 (0%)	0/118 (0%)	2/234 (1%)
Other tumors	0/468 (0%)	0/118 (0%)	0/234 (0%)
Pancreas			
Islet-cell adenoma	15/456 (3%)	3/115 (3%)	2/222 (1%)*
Islet-cell carcinoma	0/222 (0%)	0/115 (0%)	1/456 (1%)
Thyroid			
C-cell adenoma	6/440 (1%)	1/106 (1%)	4/215 (2%)
C-cell carcinoma	1/440 (<1%)	0/106 (0%)	1/215 (<1%)
Other tumors	2/440 (1%)	0/106 (0%)	0/215 (0%)
Parathyroid, ademona	6/280 (2%)	1/61 (1%)	1/141 (1%)
G.I. tract, all tumors	5/472 (1%)	1/119 (1%)	4/237 (2%)
Pituitary, all tumors	2/285 (1%)	0/79 (0%)	0/149 (0%)
Kidney, all tumors	1/472 (<1%)	1/119 (0%)	0/236 (0%)
Liver, all tumors	0/472 (0%)	0/118 (0%)	0/234 (0%)
Leukemia or malignant lymphoma	7/472 (1%)	2/119 (2%)	3/2236 (1%)
Hemangioma or hemangiosarcoma	2/472 (<1%)	1/119 (1%)	3/237 (1%)
Uterus, all tumors	8/471 (2%)	2/119 (1%)	1/236 (<1%)
All other tumors	6/472 (1%)	1/119 (1%)	2/237 (1%)

*$p < 0.05$ decrease relative to pooled controls (life table and incidental tests).

Table 12. Incidence of primary tumors in male hamsters administered short-range chrysotile asbestos.

	Pooled controls	Short-range chrysotile controls	Short-range chrysotile
Animals with primary tumors	95/472 (20%)	21/115 (18%)	64/233 (27%)[a]
Skin or subcutaneous tissue, all tumors	2/472 (<1%)	0/115 (0%)	0/233 (0%)
Lung or trachea, all tumors	0/470 (0%)	0/115 (0%)	0/231 (0%)
Adrenal			
Cortical adenoma	25/466 (5%)	7/115 (6%)	26/229 (11%)[b]
Cortical carcinoma	13/466 (3%)	3/115 (3%)	8/229 (3%)
Pheochromocytoma	13/466 (3%)	2/115 (2%)	4/229 (2%)
Other tumors	6/466 (1%)	0/115 (0%)	1/229 (<1%)
Pancreas			
Islet-cell adenoma	20/445 (4%)	2/111 (2%)	15/218 (7%)
Islet-cell carcinoma	1/445 (<1%)	1/111 (1%)	0/218 (0%)
Thyroid			
C-cell adenoma	7/428 (2%)	3/109 (3%)	3/207 (1%)
C-cell carcinoma	3/428 (1%)	1/109 (1%)	1/207 (<1%)
Other tumors	1/428 (<1%)	0/109 (0%)	0/207 (0%)
Parathyroid, adenoma	2/271 (1%)	0/72 (0%)	3/132 (2%)
G.I. tract, all tumors	6/472 (1%)	2/115 (2%)	0/233 (0%)
Pituitary, all tumors	0/322 (0%)	0/84 (0%)	0/159 (0%)
Kidney, all tumors	4/470 (1%)	0/115 (0%)	3/232 (1%)
Liver, all tumors	0/470 (0%)	0/115 (0%)	0/232 (0%)
Leukemia or malignant lymphoma	8/472 (2%)	2/115 (2%)	3/233 (1%)
Hemangioma or hemangiosarcoma	5/472 (1%)	0/115 (0%)	4/233 (2%)
All other tumors	5/472 (1%)	1/115 (1%)	3/233 (1%)

[a]$p < 0.05$ vs. pooled controls.
[b]$p = 0.152$ (life table); $p = 0.065$ (incidental tumor test) and $p = 0.019$ (Fisher's exact test) vs. pooled controls.

Table 13. Incidence of primary tumors in female hamsters administered short-range chrysotile asbestos.

	Pooled controls	Short-range chrysotile controls	Short-range chrysotile
Animals with primary tumors	66/472 (14%)	19/114 (17%)	28/228 (12%)
Skin or subcutaneous tissue, all tumors	0/472 (0%)	0/114 (0%)	3/228 (1%)
Lung or trachea, all tumors	0/471 (0%)	0/114 (0%)	0/228 (0%)
Adrenal			
Cortical adenoma	15/468 (3%)	4/112 (4%)	8/226 (4%)
Cortical carcinoma	0/468 (0%)	0/112 (0%)	0/226 (0%)
Pheochromocytoma	0/468 (0%)	0/112 (0%)	3/226 (1%)
Other tumors	0/468 (0%)	0/112 (0%)	1/226 (<1%)
Pancreas			
Islet-cell adenoma	15/456 (3%)	2/109 (2%)	2/217 (1%)[a]
Islet-cell carcinoma	1/456 (<1%)	1/109 (1%)	0/217 (0%)
Thyroid			
C-cell adenoma	6/440 (1%)	2/107 (2%)	0/214 (0%)
C-cell carcinoma	1/440 (<1%)	1/107 (0%)	0/214 (0%)
Other tumors	2/440 (<1%)	2/107 (2%)	0/214 (0%)
Parathyroid, adenoma	6/280 (2%)	3/68 (4%)	3/139 (2%)
G.I. tract, all tumors	5/472 (1%)	1/114 (1%)	1/228 (<1%)
Pituitary, all tumors	2/285 (1%)	0/77 (0%)	0/132 (1%)
Kidney, all tumors	1/472 (<1%)	0/114 (0%)	0/228 (0%)
Liver, all tumors	0/472 (0%)	0/114 (0%)	0/228 (0%)
Leukemia or malignant lymphoma	7/472 (1%)	2/114 (2%)	2/228 (1%)
Hemangioma or hemangiosarcoma	2/472 (<1%)	0/114 (0%)	1/228 (<1%)
Uterus, all tumors	8/471 (2%)	3/113 (3%)	5/226 (2%)
All other tumors	6/472 (1%)	3/114 (3%)	3/228 (1%)

[a]$p < 0.05$ decrease relative to pooled controls (life table and incidental tumor test).

Table 14. Incidence of primary tumors in male hamsters administered intermediate-range chrysotile asbestos.

	Pooled controls	Intermediate-range chrysotile controls	Intermediate-range chrysotile
Animals with primary tumors	95/472 (20%)	26/116 (22%)	78/245 (32%)[a,b]
Skin or subcutaneous tissue, all tumors	2/472 (<1%)	1/116 (1%)	0/245 (0%)
Lung or trachea, all tumors	0/470 (0%)	0/116 (0%)	1/244 (<1%)
Adrenal			
Cortical adenoma	25/466 (5%)	7/115 (6%)	24/244 (10%)[c]
Cortical carcinoma	13/466 (3%)	3/115 (3%)	7/244 (3%)
Pheochromocytoma	13/466 (3%)	5/115 (4%)	11/244 (5%)
Other tumors	6/466 (1%)	3/115 (3%)	1/244 (<1%)
Pancreas			
Islet-cell adenoma	20/445 (4%)	7/110 (6%)	15/226 (7%)
Islet-cell carcinoma	1/445 (<1%)	0/110 (0%)	1/226 (<1%)
Thyroid			
C-cell adenoma	7/428 (2%)	3/106 (3%)	5/216 (2%)
C-cell carcinoma	3/428 (1%)	1/106 (1%)	4/216 (2%)
Other tumors	1/428 (<1%)	0/106 (0%)	1/216 (<1%)
Parathyroid, adenoma	2/271 (1%)	1/71 (1%)	4/138 (3%)
G.I. tract, all tumors	6/472 (1%)	1/116 (1%)	3/245 (1%)
Pituitary, all tumors	0/322 (0%)	0/77 (0%)	0/182 (0%)
Kidney, all tumors	4/470 (1%)	2/116 (2%)	1/245 (<1%)
Liver, all tumors	0/470 (0%)	0/116 (0%)	0/244 (0%)
Leukemia or malignant lymphoma	8/472 (2%)	1/116 (1%)	10/245 (4%)
Hemangioma or hemangiosarcoma	5/472 (1%)	0/116 (0%)	1/242 (<1%)
All other tumors	5/472 (1%)	0/116 (0%)	2/245 (1%)

[a]$p < 0.01$ vs. pooled controls.
[b]$p < 0.05$ vs. intermediate-range chrysotile controls.
[c]$p < 0.05$ vs. pooled controls.

Table 15. Incidence of primary tumors in female hamsters administered intermediate-range chrysotile asbestos.

	Pooled controls	Intermediate-range chrysotile controls	Intermediate-range chrysotile
Animals with primary tumors	66/472 (14%)	17/119 (14%)	39/244 (16%)
Skin or subcutaneous tissue, all tumors	0/472 (0%)	0/119 (0%)	2/244 (1%)
Lung or trachea, all tumors	0/471 (0%)	0/119 (0%)	0/243 (0%)
Adrenal			
Cortical adenoma	15/468 (3%)	6/118 (5%)	18/234 (8%)[a]
Cortical carcinoma	0/468 (0%)	0/118 (0%)	1/234 (<1%)
Pheochromocytoma	0/468 (0%)	0/118 (0%)	1/234 (<1%)
Other tumors	0/468 (0%)	0/118 (0%)	0/234 (0%)
Pancreas			
Islet-cell adenoma	15/456 (3%)	5/116 (6%)	4/236 (2%)
Islet-cell carcinoma	1/456 (<1%)	0/116 (0%)	0/236 (0%)
Thyroid			
C-cell adenoma	6/440 (1%)	3/115 (3%)	2/223 (1%)
C-cell carcinoma	1/440 (<1%)	0/115 (0%)	0/223 (0%)
Other tumors	2/440 (<1%)	0/115 (0%)	1/223 (<1%)
Parathyroid, adenoma	6/280 (2%)	1/77 (1%)	1/148 (1%)
G.I. tract, all tumors	5/472 (1%)	2/119 (2%)	1/244 (<1%)
Pituitary, all tumors	2/285 (1%)	2/67 (3%)	2/164 (1%)
Kidney, all tumors	1/472 (<1%)	1/119 (1%)	0/243 (0%)
Liver, all tumors	0/472 (0%)	0/119 (0%)	0/234 (0%)
Leukemia or malignant lymphoma	7/472 (1%)	0/119 (0%)	2/244 (1%)
Hemangioma or hemangiosarcoma	2/472 (<1%)	0/119 (0%)	1/244 (<1%)
Uterus, all tumors	8/471 (2%)	1/119 (1%)	7/240 (3%)
All other tumors	6/472 (1%)	0/119 (0%)	2/244 (1%)

[a]$p < 0.05$ vs. pooled controls.

Table 16. Incidence of primary tumors in male hamsters administered 1,2-dimethylhydrazine dihydrochloride (DMH) or intermediate-range chrysotile asbestos and DMH.

	Pooled controls	DMH and intermediate-range chrysotile controls	DMH	DMH and intermediate-range chrysotile
Animals with primary tumors	95/472 (20%)	27/119 (23%)	29/127 (23%)	51/173 (29%)[a]
Skin or subcutaneous tissue, all tumors	2/472 (<1%)	1/119 (1%)	0/127 (0%)	1/173 (1%)
Lung or trachea, all tumors	0/470 (0%)	0/119 (0%)	0/126 (0%)	0/173 (0%)
Adrenal				
Cortical adenoma	25/466 (5%)	3/117 (3%)	3/127 (2%)	8/171 (5%)
Cortical carcinoma	13/466 (3%)	4/117 (4%)	2/127 (2%)	7/171 (4%)
Pheochromocytoma	13/466 (3%)	3/117 (3%)	4/127 (3%)	6/171 (4%)
Other tumors	6/466 (1%)	2/117 (2%)	0/127 (0%)	1/171 (1%)
Pancreas				
Islet-cell adenoma	20/445 (4%)	8/110 (7%)	6/114 (5%)	10/167 (6%)
Islet-cell carcinoma	1/445 (<1%)	0/110 (0%)	0/114 (0%)	1/167 (1%)
Thyroid				
C-cell adenoma	7/428 (2%)	0/107 (0%)	2/118 (2%)	3/163 (2%)
C-cell carcinoma	3/428 (1%)	0/107 (0%)	0/118 (0%)	1/163 (1%)
Other tumors	1/428 (<1%)	1/107 (1%)	0/118 (0%)	0/163 (0%)
Parathyroid, adenoma	2/271 (1%)	1/64 (2%)	0/81 (0%)	2/118 (2%)
G.I. tract, all tumors	6/472 (1%)	2/119 (2%)	3/127 (2%)	4/173 (2%)
Pituitary, all tumors	0/322 (0%)	0/80 (0%)	0/87 (1%)	2/123 (2%)
Kidney, all tumors	4/470 (1%)	1/119 (1%)	0/127 (0%)	0/173 (0%)
Liver, all tumors	0/470 (0%)	0/119 (0%)	2/127 (2%)	1/173 (1%)
Leukemia or malignant lymphoma	8/472 (2%)	4/119 (4%)	7/127 (6%)[b]	8/173 (5%)
Hemangioma or hemangiosarcoma	5/472 (1%)	3/119 (3%)	2/127 (2%)	2/173 (1%)
All other tumors	5/472 (1%)	3/119 (3%)	1/127 (1%)	4/173 (2%)

[a]$p = 0.257$ (life table); $p = 0.038$ (incidental tumor test); $p = 0.009$ (Fisher's exact test) vs. pooled controls.
[b]$p < 0.05$ vs. pooled controls.

Table 17. Incidence of primary tumors in female hamsters administered 1,2-dimethylhydrazine dihydrochloride (DMH) or intermediate-range chrysotile asbestos and DMH.

	Pooled controls	DMH and intermediate-range chrysotile controls	DMH	DMH and intermediate-range chrysotile
Animals with primary tumors	66/472 (14%)	15/120 (12%)	15/122 (12%)	19/161 (12%)
Skin or subcutaneous tissue, all tumors	0/472 (0%)	0/120 (0%)	1/122 (1%)	0/161 (0%)
Lung or trachea, all tumors	0/471 (0%)	0/119 (0%)	0/122 (0%)	1/160 (1%)
Adrenal				
Cortical adenoma	15/468 (3%)	3/120 (2%)	2/120 (2%)	6/158 (4%)
Cortical carcinoma	0/468 (0%)	0/120 (0%)	0/120 (0%)	2/158 (1%)
Pheochromocytoma	0/468 (0%)	0/120 (0%)	0/120 (0%)	0/158 (0%)
Other tumors	0/468 (0%)	0/120 (0%)	0/120 (0%)	0/158 (0%)
Pancreas				
Islet-cell adenoma	15/456 (3%)	5/116 (4%)	2/119 (2%)	4/149 (3%)
Islet-cell carcinoma	1/456 (<1%)	0/116 (0%)	0/119 (0%)	1/149 (0%)
Thyroid				
C-cell adenoma	6/440 (1%)	0/112 (0%)	0/108 (0%)	0/141 (0%)
C-cell carcinoma	1/440 (<1%)	1/112 (1%)	0/108 (0%)	0/141 (0%)
Other tumors	2/440 (<1%)	1/112 (0%)	0/108 (0%)	0/141 (0%)
Parathyroid, adenoma	6/280 (2%)	1/74 (1%)	2/57 (4%)	0/91 (0%)
G.I. tract, all tumors	5/472 (1%)	1/120 (1%)	2/122 (2%)	0/161 (0%)
Pituitary, all tumors	2/285 (1%)	0/62 (0%)	0/59 (0%)	0/109 (0%)
Kidney, all tumors	1/472 (<1%)	0/120 (0%)	0/122 (0%)	0/161 (0%)
Liver, all tumors	0/472 (0%)	0/119 (0%)	0/121 (0%)	0/161 (0%)
Leukemia or malignant lymphoma	7/472 (1%)	3/120 (2%)	2/122 (2%)	3/161 (2%)
Hemangioma or hemangiosarcoma	2/472 (<1%)	1/120 (1%)	0/122 (0%)	1/161 (1%)
Uterus, all tumors	8/471 (2%)	2/120 (2%)	2/116 (2%)	2/156 (1%)
All other tumors	6/472 (1%)	1/120 (1%)	2/122 (2%)	2/161 (1%)

Table 18. Incidence of gastrointestinal tract tumors in hamsters administered amosite asbestos.

	Pooled controls		Amosite controls		Amosite-treated	
	M	F	M	F	M	F
Stomach (number examined)	(464)	(468)	(120)	(117)	(247)	(236)
Squamous cell papilloma	3	0	1	0	4	0
Papilloma	0	0	0	0	0	3
Small intestine (number examined)	(467)	(469)	(120)	(117)	(246)	(236)
Adenoma	1	0	0	0	0	0
Adenocarcinoma	1	0	0	0	0	0
Large intestine (number examined)	(464)	(468)	(118)	(116)	(246)	(235)
Adenoma	0	1	0	0	0	0
Papillary adenoma	0	0	0	0	1	0
Adenocarcinoma	0	1	0	0	0	0
Lipoma	0	1	0	0	0	0
Rectum (number examined)	(472)	(272)	(122)	(119)	(248)	(237)
Adenoma	1	1	0	1	0	0
Adenomatous polyp	0	0	0	0	1	0
Fibroma	0	1	0	0	0	0
Squamous cell papilloma	0	0	0	0	0	0

Males and females administered DMH did not show a significant ($p < 0.05$) increase in intestinal neoplasia. Nor did the intermediate-range chrysotile produce a higher rate of intestinal neoplasia in DMH-dosed animals. A summary of all gastrointestinal tumors observed in this study is given in Tables 18 and 19. None of the treated groups showed an increased rate of neoplasia in the gastrointestinal tract which was the proposed target organ.

While this study was not designed to evaluate nonneoplastic disease, noteworthy lesions were observed. None appeared to be dosage related; rather, they were consistent with lesions that are normally found in aging hamsters. It was the pathologists' opinion that the most important le-

Table 19. Incidence of gastrointestinal tract tumors in hamsters administered chrysotile asbestos.

	Pooled controls		Short-range chrysotile		Intermediate-range chrysotile		DMH		DMH and Intermediate-range chrysotile	
	M	F	M	F	M	F	M	F	M	F
Stomach (number examined)	(464)	(468)	(222)	(224)	(244)	(242)	(127)	(118)	(170)	(160)
Squamous cell papilloma	3	0	0	0	1	0	0	0	2	0
Carcinoma *in situ*	0	0	0	0	1	0	0	0	0	0
Papillary adenoma	0	0	0	0	1	1	0	0	0	0
Small intestine (number examined)	(467)	(469)	(226)	(227)	(244)	(244)	(127)	(120)	(170)	(159)
Adenoma	1	0	0	0	0	0	0	0	0	0
Adenomacarcinoma	1	0	0	0	0	0	0	0	0	0
Large intestine (number examined)	(464)	(468)	(222)	(226)	(241)	(243)	(126)	(118)	(170)	(159)
Papilloma	0	0	0	0	0	0	0	0	1	0
Adenoma	0	1	0	0	0	0	1	0	0	0
Papillary adenoma	0	0	0	0	0	0	1	0	0	0
Adenocarcinoma	0	1	0	1	0	0	0	0	0	0
Lipoma	0	1	0	0	0	0	0	0	0	0
Adenomatous polyp	0	0	0	0	0	0	0	1	0	0
Rectum (number examined)	(472)	(472)	(233)	(228)	(245)	(244)	(127)	(122)	(173)	(161)
Adenoma	1	1	0	0	0	0	0	0	0	0
Papillary adenoma	0	0	0	0	0	0	0	0	1	0
Fibrosarcoma	0	0	0	0	0	0	1	0	0	0
Squamous cell carcinoma	0	0	0	0	0	0	0	1	0	0
Fibroma	0	1	0	0	0	0	0	0	0	0

sion, responsible for many deaths, was generalized amyloidosis. The kidneys were particularly affected by diffuse accumulation of amyloid, which replaced glomeruli and infiltrated tubular interstitium to a point where the normal cortical architecture was obliterated. Other organs which showed significant accumulations of amyloid were adrenal gland, liver, spleen and the epithelium of the small intestine. Amyloid within the walls of blood vessels was observed in many tissues.

Many of the livers were cirrhotic, infiltrated with amyloid, and contained large cystic structures filled with a lightly staining proteinaceous fluid. These structures were interpreted as cystic bile ducts and are consistent with what others have termed "retention cysts." At times, these cysts were so large and/or numerous that less than half of the livers remained.

Other nonneoplastic lesions that were observed in more than 5% of the hamsters in any of the experimental groups are as follows: (1) skin, chronic dermitis; (2) lung, interstitial pneumonitis; (3) spleen, lymphoid atrophy; (4) lymph node, hyperplasia; (5) heart, atrial thrombosis; (6) gallbladder, edema and calculi; (7) stomach (nonglandular), hyperkeratosis or acanthosis; (8) colon, intussusception, inflammation; (9) urinary bladder, chronic inflammation, hyperplasia; (10) adrenal gland, cortical and medullary hyperplasia; (11) thyroid gland, follicular atrophy; (12) pituitary gland, degeneration; (13) ovary, atrophy; (14) uterus, inflammation, endometrial hyperplasia; (15) vagina, acute inflammation, squamous metaplasia.

Discussion

The clinicopathologic results in this study showed that the chronic ingestion of 1% amosite (*8*) or chrysotile (SR and IR) (*9*) asbestos in the diet did not have any adverse effect on body weight gain and survival seemed to be enhanced. An explanation for these observations is not apparent.

The major organ which showed a statistically significant ($p < 0.05$) increased rate of neoplasia was the adrenal cortex in male and female hamsters exposed to IR chrysotile asbestos and males exposed to SR chrysotile asbestos when compared with pooled controls. However, statistical significance was lost when these groups were compared to their temporal controls. It is difficult to imagine how oral asbestos, even though it is known to be absorbed through the gastrointestinal tract (*10*), could cause an increased tumor rate in the adrenal cortex without causing similar increases in tumors in other abdominal organs and tissues, i.e., gastrointestinal tract and peritoneum. For these reasons, the biologic importance of adrenal tumors in this study is doubtful. The overall increase in total primary tumors in male IR chryso-

tile hamsters can be explained primarily on the basis of an increased incidence of adrenal tumors in this group. The enhanced survival of animals in the chrysotile groups also contributed to the elevated incidence of primary tumors observed in these groups compared with controls.

The only other instance of an increased rate of neoplasia was a significant ($p < 0.05$) increase in leukemia or malignant lymphoma in male hamsters exposed to DMH when compared to pooled controls. Again, statistical significance was lost when this group was compared to its temporal control group. This finding also loses importance because it was not observed in the DMH plus IR chrysotile group.

Other such studies involving the long-term ingestion of asbestos are few. Donham et al. (*11*) reported equivocal results in F344 rats which were fed a diet containing 10% chrysotile for their lifetime. While they did not observe a statistically significant ($p < 0.05$) increase in the number of tumors in exposed animals, the authors believed that there was a trend toward increased colon lesions in general, evidence of penetration of asbestos into the colonic mucosa and possible cytotoxicity to colonic tissues and they suggested a possible relationship to peritoneal mesothelioma. Another equivocal study is that reported by Gibel et al. (*12*), who described an increase in malignant tumors in the lung, kidney, liver and reticuloendothelial system, but no increase in intestinal neoplasia in Wistar rats fed asbestos filter material (20 mg/day) for a period of 8–14 months. Cunningham et al. (*13*) reported two studies in male Wistar rats administered 1% chrysotile in the diet, one study of 24 months and one of 30 months. No increase in intestinal tumors was found compared to the control rats. Negative results were reported by Gross et al. (*14*), who fed rats a diet containing 5% chrysotile asbestos for a period of 21 months with no evidence of intestinal neoplasia.

The only oral asbestos study in hamsters was reported by Smith et al. (*15*). They exposed groups of 30 male and female hamsters via drinking water for lifetime to amosite asbestos, mine tailings, beach rock, and Lake Superior drinking water. They did not observe adverse effects on body weight or survival time in any of the groups. A peritoneal mesothelioma, one pulmonary carcinoma, and two early squamous cell carcinomas of the nonglandular stomach were found in the hamsters exposed to amosite, but the incidence was not statistically significant ($p < 0.05$). Their other studies were considered negative. They concluded that the study was essentially negative.

Except for those by Donham et al. (*11*) and Smith et al. (*15*), these studies were conducted with relatively small numbers of animals. Also, some were conducted for periods of time insufficient for adequately testing the carcinogenic potential of ingested asbestos.

The results of the combination study (IR chrysotile plus DMH) also did not yield a significant increase in tumors above the background level observed in the DMH group alone or in the untreated control group. The DMH failed to yield a background level of intestinal tumors high enough to provide a valid test of the cocarcinogenic potential of chrysotile asbestos. For this reason, a cocarcinogenic potential of oral asbestos should be considered untested. However, the DMH plus chrysotile provides an additional IR chrysotile group for comparative purposes.

It is not clear why the DMH-dosed group of hamsters failed to show an increased incidence of intestinal neoplasia. The pilot study suggested that this dose of DMH should have caused an incidence of approximately 15%. DMH solutions rapidly decompose if they are at room temperature or if they are not properly buffered.

The only long-term study designed to determine the cocarcinogenic potential of asbestos was reported by Ward et al. (*16*). They administered 1 mg amosite asbestos in saline by gavage to 6-week-old F344 rats three times per week for 10 weeks. Once per week during this same period, half of the rats received subcutaneous injections of 7.4 mg/kg azoxymethane (AOM), a known intestinal carcinogen in animals. All surviving rats were killed at 94–95 weeks of age. They reported an intestinal tumor incidence of 66.7% for AOM alone, 77.1% for amosite plus AOM, and 32.6% for amosite alone. The authors concluded that while amosite did not significantly add to the incidence of AOM-induced intestinal neoplasia, amosite alone caused a relatively high rate of intestinal neoplasia. However, there was no untreated control group with which to compare the treated groups. These results should as well be viewed with some suspicion because the authors also reported a 14% incidence of Zymbal gland tumors in the rats exposed to amosite alone. The historical rate of Zymbal gland tumors in the Bioassay Program is 0.34%, indicating that this is an uncommon spontaneous tumor. However, AOM is known to induce Zymbal gland tumors with a single dose of 5.1 mg/kg in male F344 rats producing a 14% incidence of tumors in this organ (*17*); in this study 5.1 mg/kg AOM also caused a 24% incidence of intestinal neoplasia. An appropriate explanation for the high incidence of Zymbal

gland tumors in the amosite group would be that those animals were inadvertently exposed to AOM. If this occurred, animals would also be expected to show a high incidence of intestinal neoplasia.

Conclusion

Under the conditions of this bioassay, amosite asbestos and short-range chrysotile and intermediate-range chrysotile asbestos were not carcinogenic when ingested by male and female Syrian golden hamsters. While there were significant increases in the rates of adrenal cortical adenomas in male and female hamsters exposed to intermediate-range chrysotile asbestos compared with pooled control groups, these incidence rates were not significantly different when compared with the temporal control groups. Additionally, the biological importance of adrenal tumors in the absence of target organ neoplasia is questionable. The cocarcinogen studies using IR chrysotile asbestos and 1,2-dimethylhydrazine dihydrochloride were considered inadequate because there was no increase in intestinal neoplasia in the DMH group.

The animal phase of this study was performed at the IIT Research Institute, Chicago, IL. This project has been funded with federal funds from the National Institute of Environmental Health Sciences (NIH), Department of Health and Human Services, Research Triangle Park, NC, under contract #NO1-ES-5-2157. Partial funding was provided by the Environmental Protection Agency under Interagency Agreement No. D70756.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Proceedings of the Joint NIEHS-EPA Conference on Biological Effects of Ingested Asbestos. Environ. Health Perspect. 9: 113–462 (1974).
2. McConnell, E. E., Wilson, R. E., Moore, J. A., and Haseman, J. K. Dose response of 1,2-dimethylhydrazine and methylazoxymethanol acetate in the F344 rat. Cancer Letters 8: 271–278 (1980).
3. Kaplan, E. L., and Meier, P. Nonparametric estimation from incomplete observations. J. Am. Statist. Assoc. 53: 457–481 (1958).
4. Cox, D. R. Regression models and life tables. J. Roy Statist. Soc. 334: 187–220 (1972).
5. Mantel, N., and Haenszel, W. Statistical aspects of the analysis of data from retrospective studies of disease. J. Natl. Cancer Inst. 22: 719–748 (1959).
6. Peto, R., Pike, M., Day, N., Gray, R., Lee, P., Parish, S., Peto, J., Richard, S., and Wahrendorf, J. Guidelines for simple, sensitive significant tests for carcinogenic effects in long-term animal experiments. International Agency for Research Against Cancer. Monographs on the Long-Term and Short-Term Screening Assays for Carcinogens: A Critical Appraisal. World Health Organization Geneva, Supplement Vol. 2, 1980, pp. 311–426.
7. Gart, J., Chu, K., and Tarone, R. Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. J. Natl. Cancer Inst. 62: 957–974 (1979).
8. National Toxicology Program. NTP Technical report on carcinogenesis bioassay of amosite asbestos in Syrian golden hamsters. NTP TR 249, Department of Health and Human Services, Research Triangle Park, NC, 1982.
9. National Toxicology Program. NTP Technical report on carcinogenesis bioassay of chrysotile asbestos in Syrian golden hamsters. NTP TR 246, Department of Health and Human Services, Research Triangle Park, NC, 1982.
10. Cook, P. M., and Olson, G. F. Ingested mineral fibers: elimination in human urine. Science 204: 195–198 (1979).
11. Donham, K. J., Berg, J. W., Will, L. A., and Leininger, J. R. The effects of long term ingestion of asbestos on the colon of F344 rats. Cancer 45: 1073–1084 (1980).
12. Gibel, W., Lohs, K. H., Horn, K. H., Wildner, G. P., and Hoffman, F. Investigation into a carcinogenic effect of asbestos filter material following oral intake in experimental animals. Arch. Geschwulstforsch. 46: 437–442 (1976).
13. Cunningham, H. M., Moodie, C. A., Lawrence, G. A., and Pontefract, R. D. Chronic effects of ingested asbestos in rats. Arch. Environ. Contam. Toxicol. 6: 507–513 (1977).
14. Gross, P., Harley, R. A., Swinberne, L. M., Davis, J. M. G., and Green, W. B. Ingested mineral fibres, do they penetrate tissue or cause cancer? Arch. Environ. Health 29: 341–347 (1974).
15. Smith, W. E., Hubert, D. D., Sobel, H. J., Peters, E. T., and Doerfler, T. E. Health in experimental animals drinking water with and without amosite and other mineral particles. J. Environ. Pathol. Toxicol. 3: 277–300 (1980).
16. Ward, J. M., Frank, A. L., Wenk, M., Devor, D., and Tarone, R. E. Ingested asbestos and intestinal carcinogenesis in F344 rats. J. Environ. Pathol. Toxicol. 3: 301–312 (1980).
17. Ward, J. M. Dose response to a single injection of azoxymethane in rats. Vet. Pathol. 12: 165–177 (1975).

Environmental Health Perspectives
Vol. 53, pp. 27–44, 1983

Chronic Effects of Dietary Exposure to Amosite Asbestos and Tremolite in F344 Rats

by Ernest E. McConnell,* Henry A. Rutter,† Borge M. Ulland† and John A. Moore*

Carcinogenesis bioassays of blocky (nonfibrous) tremolite and amosite asbestos alone or in combination with the intestinal carcinogen 1,2-dimethylhydrazine dihydrochloride (DMH) were conducted with male and female Fischer 344 rats. The minerals were administered at a concentration of 1% in pelleted diet for the entire lifetime of the rats starting with the dams of the test animals. One group of amosite rats also received chrysotile asbestos via gavage during lactation. Group sizes varied from 100 to 250 animals.

The offspring from mothers exposed to tremolite or amosite asbestos were smaller at weaning than those from untreated mothers and remained smaller throughout their life. The administration of dimethylhydrazine (DMH) did not affect body weight gain, either in amosite-exposed or nonexposed animals. Survival was comparable in the tremolite and control groups. The amosite-exposed rats showed enhanced survival compared to the untreated controls. DMH exposure reduced survival by approximately one year, although the amosite plus DMH groups survived slightly better than the DMH alone groups.

No toxicity or increase in neoplasia was observed in the tremolite-exposed rats compared to the controls. Significant increases ($p < 0.05$) in the rates of C-cell carcinomas of the thyroid and monocytic (mononuclear cell) leukemia in male rats were observed in amosite-exposed groups. However, the biological significance of the C-cell carcinomas in relation to amosite asbestos exposure is discounted because of a lack of significance when C-cell adenomas and carcinomas were combined and the positive effect was not observed in the amosite plus preweaning gavage group. The biological significance of an increased incidence of mononuclear cell leukemia is questionable, because of a lack of statistical significance in the amosite group when evaluated using life table analysis, lack of significance when compared to the tremolite control group, and the fact that no toxic or neoplastic lesions were observed in the target organs, i.e., gastrointestinal tract and mesothelium.

DMH caused a high rate of (62–74%) of intestinal neoplasia in amosite and nonamosite-exposed groups. Neither an enhanced carcinogenic nor protective effect was demonstrated by exposure to amosite asbestos.

Introduction

In November 1973 the National Institute of Environmental Health Sciences and the Environmental Protection Agency cosponsored a symposium on the possible biological effects of ingested asbestos. (*1*). This conference concluded that a paucity of definitive data existed concerning the effects of ingested asbestos and that specific research was needed.

A subcommittee of the DHEW Committee to Coordinate Toxicology and Related Programs was established to review existing data and to prepare a draft research protocol that would be responsive to the possible public health implication of ingested asbestos. This protocol was widely distributed for comment within and outside the government and a public meeting of the subcommittee was held on February 11, 1975. On the basis of the comments received, a revised final protocol was developed which called for the use of long-

*The National Toxicology Program, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709.

†Hazelton Laboratories America, Inc., 9200 Leesburg Pike, Vienna, VA 22180.

term animal toxicology studies to evaluate the ingestion of several minerals for carcinogenic effect. As a result, the National Toxicology Program has investigated the carcinogenic potential of the ingestion of chrysotile asbestos in hamsters and rats, amosite asbestos in hamsters and rats, crocidolite asbestos in rats and tremolite in rats. All of the studies were to encompass the lifetime of the animal, including exposure of the dams from which the test animals were derived.

Crystalline tremolite (not actually in asbestos fiber) was chosen for this study because up to 20 years ago it was a common contaminant of talc which was used in foods and pharmaceuticals. The grinding of tremolite in preparation for its intended use may result in the production of fibers which have the morphology of asbestos minerals. Stanton et al. (2), in reviewing intrapleural mineral deposition studies, speculated that the asbestos mineral hazard question may be directly related to fiber size in contrast to chemical composition. Therefore, the study of crystalline tremolite was deemed appropriate because of its past widespread exposure and the fact that it assumes fiber characteristics when ground in the processing of talc.

This report represents the results of those studies undertaken to determine the effects of tremolite or amosite asbestos in the diet fed to Fischer 344 rats. In addition, the study was designed to determine if the feeding of amosite asbestos modified the response of a known intestinal carcinogen, 1,2-dimethlhydrazine dihydrochloride (DMH). Reports on chrysotile and crocidolite asbestos will be reported later.

Table 1. Fiber characteristics of amosite asbestos.

Fiber characteristics	
Surface area, m^2/g	4.13
Density, g/cm^3	3.35 ± 0.026 SD
Measurements, transmission electron microscopy	
Fiber count/g	0.3466×10^{10}
Median length (μm)	4.37
Range of length, μm	0.85 – 995
Median diameter, μm	0.72
Range of diameter, μm	0.064 – 12.4
Median fiber aspect ratio (*l*/*d*)	6.4248

Table 2. Chemical-instrumental analysis of amosite asbestos.

	Content, wt-%
Al_2O_3	0.42
CaO	0.48
FeO	34.61
Fe_2O_3	2.24
MgO	6.22
K_2O	0.30
SiO_2	50.36
Na_2O	0.03
MnO	2.66
Cr_2O_3	0.03
NiO	0.01
CO_2	0.88
H_2O-	0.15
H_2O+	2.30
Benzene extracted organics	0.021

Materials and Methods

Test Materials

Asbestos is a general term applied to certain natural silicates when they appear in a fibrous

Table 3. Particle size distribution of amosite asbestos by particle number: SEM.

	Length interval, μm						
	0–1.99	2–3.99	4–5.99	6–7.99	8–9.99	10–19.99	20–39.99
Amosite mean width, μm	0.28	0.38	0.45	0.45	0.48	0.52	0.51
Amosite particles per interval	57	126	88	78	52	181	184
Total amosite particles, %	5.6	12.3	8.6	7.6	5.1	17.7	18.0
Cumulative % amosite	5.6	17.9	26.5	34.1	39.2	56.9	74.9
Amosite, vol-%[a]	–	0.1	0.3	0.4	0.4	2.4	5.0
Cumulative volume-% amosite	–	0.1	0.4	0.8	1.2	3.6	8.6
Number of other particles	11	8	1	0	1	1	0
Amosite particles per length interval by aspect ratio, %							
1:1–2.9:1	12	0	0	0	0	0	0
3:1–4.9:1	34	10	6	5	2	0	0
5:1–9.9:1	43	52	23	14	4	1	1
10:1–19.9:1	11	34	52	38	40	21	1
20:1–49.9:1	0	4	18	41	54	64	30
50:1–99.9:1	0	0	1	2	0	12	55
100:1–199:1	0	0	0	0	0	2	12
200:1–499:1	0	4	0	0	0	0	1
>500:1	0	0	0	0	0	0	0

[a]Calculated from particle number data, assuming rectangular cross section with third dimension equal to 1/2 measured width.

form. Amosite is a fibrous member of the amphibole mineral group, its chemical structure is $(Fe^{2+}Mg)_7Si_8O_{22}(OH)_2$. Mineral and fiber characteristics of amosite are shown in Tables 1–3.

The amosite sample identified as S-33 was purchased by the Bureau of Mines from the Atlas Asbestos Company, Montreal, Quebec, Canada. This material is from a mine in the area known as Penge, in the Transvaal, Republic of South Africa. Not a proper mineral name, amosite is a term used to describe the material from asbestos mines in South Africa. To develop homogeneity of the sample the amosite was processed by a single pass through an air jet mill.

The temolite sample used in this study was obtained from a single lense from the Governeur Talc Company, Governeur, NY. This 1200-lb lense was taken from the 500 ft level, American vein, No. 4 footwall stope, lower portion of the footwall bedding. The lense was crushed in a Denver Jaw Crusher and then to minus 14 mesh in a roll crusher. This material was then wheeler-milled at 204°C and bagged in 50-lb Kraft bags. The final particle size was nominal minus 325 mesh. To develop homogeneity of the sample, approximately 960 lb tremolite was blended in a 10 ft^3 V-type blender. Mineral and fiber characteristics of tremolite are shown in Tables 4 and 5.

After final blending the samples were weighed to 25 ± 0.5 lb and placed in fiberboard drums. These drums were shipped to a special warehouse at Research Triangle Park, NC. Each drum received a color marking unique to the mineral type. Homogeneity of the samples was verified by fluorescent X-ray spectrography for samples collected from six randomly selected drums. No significant differences were detected.

The homogeneity of the samples and the physical and chemical properties of the materials were characterized by the Bureau of Mines, U.S. Department of the Interior (Supt. of Documents No. I 28.23:8452) and by the Fine Particle Laboratories, Illinois Institute of Technology Research In-

Table 4. Fiber characteristics and chemical-instrumental analysis of tremolite.

Fiber characteristics	
Surface area, m^2/g	5.2 ± 0.5
Density, g/cm^3	2.91 ± 0.01 SD
Analyses, wt-%	
Al_2O_3	1.57
CaO	11.26
Fe_2O_3	0.27
MgO	26.71
K_2O	0.18
SiO_2	54.00
Na_2O	0.80
TiO_2	0.03
MnO	0.05
Li_2O	0.02
SnO	0.01
SrO	0.03
Bi_2O_3	0.01
CO_2	0.78
H_2O-	0.24
H_2O+	3.73
Benzene-extracted organics	0.003

Table 5. Particle size distribution for tremolite by particle number: SEM.

	Length interval, μm										
	0–0.99	1–1.99	2–2.99	3–3.99	4–4.99	5–5.99	6–6.99	7–7.99	8–8.99	9–9.99	>10
Mean width, μm	0.48	0.88	0.97	1.51	2.05	2.19	2.79	3.29	2.96	3.13	5.22
Number of particles per interval	59	291	194	106	53	40	31	19	9	13	58
% of all particles per interval	6.8	33.4	22.3	12.2	6.1	4.6	3.6	2.2	1.0	1.4	6.4
Cumulative % of all particles	6.8	40.2	62.5	74.7	80.8	85.4	89.0	91.2	92.2	93.6	100
Tremolite particles per interval	34	197	128	83	38	27	23	15	9	12	49
% of tremolite particles	5.5	32.0	20.8	13.5	6.2	4.4	3.7	2.4	1.5	2.0	8.0
Cumulative % tremolite	5.5	37.5	58.3	71.8	78.0	82.4	86.1	88.5	9.0	92.0	100
Talc-serpentine particles per interval	9	72	53	19	11	9	8	4	0	1	7
Other particles per interval	16	22	10	4	2	4	0	0	0	0	2
Tremolite particles per length interval, by aspect ratio, %[a,b]											
1:1–2.9:1	100	92	75	67	76	67	65	66	67	30	35
3:1–4.9:1	0	8	22	29	18	30	30	20	22	35	37
5:1–9.9:1	0	0	3	4	6	3	5	7	11	35	18
10:1–19:1	0	0	0	0	0	0	0	7	0	0	4
20:1–49:1	0	0	0	0	0	0	0	0	0	0	4
50:1–99:1	0	0	0	0	0	0	0	0	0	0	0
100:1–199:1	0	0	0	0	0	0	0	0	0	0	2

[a]Data for aspect ratio obtained from a second set of measurements.
[b]Total particles = 871, total tremolite = 615, total talc-serpentine = 193, and total other = 63.

stitute, Chicago, IL (Special Report and Addendum on project L6085, contract N01-ES-5-3157). Copies of these reports are available upon request from the National Toxicology Program.

Test Diets

The feed used was NIH-31 open formula rodent diet prepared by Zeigler Brothers, Inc., Gardner, PA. Tremolite or amosite asbestos was incorporated to a level of 1% by weight into the test diet. All feed was pelleted with a Sprout-Waldron pelleter; the pellets were of oval configuration, 3/8 in. by 3/4 in. in size. Pelleted feed was packaged in 25-lb aliquots in standard paper feedbags which were color coded to minimize the occurrence of feeding errors at the test laboratory.

Each lot of blended feed was analyzed for tremolite or amosite asbestos concentrations, pesticide contamination and nutrient content.

1% Chrysotile (Medium Range) Gavage.* The required amount of chrysotile (medium range), a gray powder with lumps, was weighed on a Mettler balance and placed in a beaker. Sterile water (for injection) was added to obtain the desired concentration and the suspension was then mixed in a magnetic stirrer for a short period of time. The suspension was administered by gavage, at a dose level of 0.47 mg/g of body weight, to the amosite and preweaning gavage (PWG) animals from birth to weaning (21 days).

Source and Specifications of Test Animals

Parental Generation (F_0). Weanling Fischer 344 (caesarean-derived) rats, which were barrier sustained and specific pathogen-free, were purchased from Charles River Breeding Laboratories, Inc., Wilmington, MA. These animals constituted the F_0 generation.

On arrival, animals were taken directly to the quarantine area and acclimated to laboratory conditions for approximately 2 weeks. At 24 hr after the animals arrived, eight animals of each sex were selected, sacrificed, and pathogen burden was determined for each animal. Pathogens examined for included ectoparasites, intestinal parasites, and bacteria. Serological tests were conducted for viruses. After approximately 2 months of quarantine the rats, both males and females, were randomized and divided into test groups by a computerized randomization process and placed on the appropriate designated diets.

*Animals were to receive 1% amosite, but were inadvertently gavaged with 1% chrysotile.

After at least 7 days exposure to the appropriate diets, the rats were placed in breeding cages (one male to two females). During the breeding period, the rats continued to be fed the designated diets; 20 days later (on the average), females were separated and housed individually in polycarbonate cages (Hazleton Systems, Aberdeen, MD). Males were removed from the breeding cages and re-housed two per cage.

Filial Generation (F_1). The F_0 females were allowed to deliver their F_1 litters naturally, and these were reduced to no more than eight pups (four/sex if possible) per litter. At birth, the litters from the F_0 dams within the control and treated groups were assigned randomly to the corresponding lifetime feeding phase groups such that birth dates were equally distributed. All pups assigned to the amosite and preweaning gavage (PWG) groups were exposed to the PWG phase of the study to assure exposure to asbestos from birth to weaning.

At 21 days after birth, the pups were weaned and given a temporary number, then selected, using a random number table, to be placed in their respective groups for the lifetime feeding study. Litters in which only one sex was present were excluded from those animals to be selected. The extra weanlings were discarded.

At 8 weeks of age, 1,2-dimethylhydrazine dihydrochloride (DMH) was administered by gavage to a control group and an amosite group every 14 days for a total of five doses. Males received 7.5 mg/kg, and females 15.0 mg/kg, based on a previous pilot study (*3*) which showed that these doses produced an approximate incidence of 15% intestinal neoplasia. Concentrations of DMH in the dosing solutions were determined within one hour prior to dosing and following dosing. The results of these determinations showed that the proper concentration of DMH was present in the dosing solution and had not deteriorated during dosing.

Animal Maintenance

The control and mineral exposed rats were placed in separate rooms with monitored temperature and humidity, and a controlled light cycle (12 hr light/12 hr dark). Temperature was maintained at 74 ± 4°F and humidity at 50% ± 10%. The rats were housed three per cage in polycarbonate cages covered with nonwoven polyester filter sheets and stored on Enviro-racks. Racks and filters were changed approximately once every 2 weeks. Cages and bedding were replaced twice per week. Control and treated diets and tap water

via automatic waterers were available *ad libitum*. Two water samples were collected and submitted for asbestos analysis. Stainless steel feed containers were changed once every 2 weeks.

The incoming air in the animal rooms was filtered to remove particulate matter. Ten to fifteen changes of room air per hour were provided. Prior to initiation of the study, air samples were collected and analyzed for baseline asbestos determinations. Additional samplings were collected approximately every 6 months for analysis to assure personnel safety.

Other measures used for personnel protection included the wearing of fully protective disposable suits, gloves, boots and bouffant caps and the use of a dust/mist respirator mask. Personnel leaving the animal rooms were required to take showers. In addition, physical examinations, including pulmonary function tests and chest radiographs, were conducted at the initiation of the study, yearly thereafter, and at the end of the study.

Clinical Examinations and Pathology

Observations and Records. All animals were observed twice daily for moribund condition and mortality. Recorded weekly were individual body weights; signs of toxicity or pharmacologic effects; incidence, size and location of palpable tissue masses or nodules; and food consumption per cage.

Sacrifice and Gross Pathology. Animals were sacrificed when exhibiting any one of these conditions: palpable masses within the abdominal cavity (excluding retained testes); masses protruding from the rectum; rectal discharge of bright red fluid (an indication of the presence of a bleeding colonic or rectal neoplasm); large ulcerated masses in the area of the ears or on side of face (Zymbal gland tumors); large subcutaneous masses which have been ulcerated or infected; masses which interfere with breathing and eating or which severely hamper locomotion; huge tissue masses (>10 cm); central nervous system signs accompanied by weight loss (head tilt, circling incoordination, ataxia, paralysis); severe weight loss or emaciation; or comatose or very weak.

When the remaining animals of either the control and DMH or the corresponding amosite and DMH group of either sex was reduced to 10% of those starting the study, both groups within that sex were killed. When survival or untreated control or amosite or amosite and PWG group of either sex reached 10%, all remaining animals of these groups within that sex were killed. The tremolite-exposed groups were handled similarly. Animals were killed by exsanguination under sodium pentobarbital anesthesia (Nembutal, Abbott Laboratories, Inc., North Chicago, IL, or Diabutal, Diamond Laboratories Inc., Des Moines, IO). Final body weights were recorded and necropsies performed which included these additional procedures: blood smears taken from animals sacrificed *in extremis* or terminally sacrificed, touch preparations made from any enlarged spleen or lymphoid organ.

Since the gastrointestinal tract was considered as the target organ prior to the study, it was handled in a manner slightly different from that in standard rodent lifetime bioassays. Prior to placement in fixative, the entire esophagus was opened and examined. The stomach and cecum were opened and pinned with the exterior surface adjacent to paper; 2-cm lengths of duodenum and ileum and two portions of jejunum were placed unopened in fixative. The remaining small intestine was opened and washed gently with saline and the mucosal surface was then examined carefully using transillumination on a radiograph viewing box. Suspect lesions were processed separately and identified individually as to location. Likewise, the entire colon with anus was opened, examined, and placed on cardboard (serosal surface down) prior to fixation. The size and location of masses were recorded. Masses greater than 1 mm in diameter were removed as separate specimens for processing. After fixation and prior to embedding, the colon was "carpet-rolled" starting at the proximal end, with the mucosal surface inward.

All tissues were fixed in 10% neutral buffered formalin, sectioned, and stained with hematoxylin and eosin. Tissues/organs examined microscopically were: tissue masses, the above-mentioned portions of gastrointestinal tract, mesenteric, celiac, iliocolonic, renal, iliac, mandibular, cervical, pancreatic and bronchial lymph nodes, mammary gland, salivary gland, thigh muscle, bone marrow (sternum), nasal cavity with turbinates, larynx, trachea, lungs and bronchi, heart, thyroid, parathyroid, liver, pancreas, spleen, kidneys, adrenal glands, urinary bladder, seminal vesicles/prostate, testes/epididymus, ovaries/uterus, brain, pituitary gland, eyes and spinal cord.

Data Recording and Statistical Methods

The individual animal pathology data on this experiment were recorded in the computerized

carcinogenesis bioassay data system. The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, and individual pathologic results.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (*4*). Animals were statistically censored as of the time that they died of other than natural causes or were missing; animals dying from natural causes were not statistically censored. Differences in survival were evaluated by Cox's (*5*) life table method.

The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., leukemia), the denominators consist of the numbers of animals necropsied.

For the statistical analyses of tumor incidence data, two methods of adjusting for intercurrent mortality were employed. Each used the classical methods of combining contingency tables developed by Mantel and Haenszel (*6*). The first method of analysis assumed that all tumors of a given type were fatal; i.e., they caused the death of the animal, either directly or indirectly. According to this approach, the proportions of tumor-bearing animals in the treated and control groups were compared at each point in time at which an animal died with a particular tumor. The denominators of these proportions were the total number of animals at risk in each group. These results were then combined by the Mantel-Haenszel methods to obtain an overall probability (p) value. This method of adjusting for intercurrent mortality is Cox's life table method (*5*).

The second method of analysis assumed that all tumors of a give type were "incidental"; i.e., they were merely observed at autopsy in animal dying of an unrelated cause. According to this approach, the proportions of male and female rats found to have tumors in treated and control groups were compared in each of five time intervals: 0-60 weeks, 61-86 weeks, 87-112 weeks, 113-126 weeks and beyond 126 weeks. The denominators of these proportions were the number of animals actually autopsied during the time interval. The individual time interval comparisons were then combined by the previously described methods to obtain a single overall result (*7*). For comparisons involving groups receiving DMH (which showed markedly reduced survival), somewhat shorter time intervals were utilized for the incidental tumor test: 0-52 weeks, 53-78 weeks, 79-92 weeks, 93-116 weeks (males), 93-102 weeks (females), beyond 116 weeks (males) and beyond 102 weeks (females).

In addition to these tests, one other set of statistical analyses was carried out for each primary tumor: the Fisher exact test based on the overall proportion of tumor-bearing animals (*8*). All reported p values are one-sided. Except where noted, the three alternative analyses gave similar results.

Results

Establishment of Test Groups

The experiment was designed to evaluate the effects of orally ingested tremolite or amosite asbestos during the entire life of the animal, starting from the time the rats were able to eat solid food. For this reason, the mated female rats had been on the test diets for approximately 12 weeks when the first litters were born. To minimize the chance that the mothers would reject or cannibalize their young, the litters were not handled during lactation, except for the group receiving asbestos via preweaning gavage.

Litter size and survivability of offspring were unaffected by the presence of amosite in the diets. The average number of live fetuses born to tremolite-exposed dams was 7.6 versus 7.8 for the control groups. The average number of live fetuses born to amosite-exposed dams was 8.5 versus 7.7 for the control groups. Significant mortality was induced in those pups which received the preweaning asbestos gavage (PWG). The average size of the litters in this group was 3.4 at weaning compared to 7.5 in the non-PWG amosite group. The average weight at birth of the tremolite-exposed pups was 4.7 g versus 4.8 g for the controls. The average weight at birth of the amosite-exposed pups was 4.7 g versus 4.8 g for the controls. The tremolite-exposed offspring were slightly smaller at weaning, 22.8 g versus 26.3 g (control). The amosite-exposed offspring were also slightly smaller at weaning, 23.2 g versus 27.4 g (control).

A summary of groups, number of animals and diets for the filial (F_1) animals is presented in Table 6.

Table 6. Summary of distribution and diets: a lifetime feeding study of tremolite or amosite asbestos in rats.

Test group	No. of animals		% of diet	DMH, mg/kg[a]	
	Male	Female		Male	Female
Control	118	118	0		
Tremolite	250	250	1		
Control	117	117	0	–	–
DMH	125	125	0	7.5	15.0
Amosite	250	250	1	–	–
Amosite + DMH	175	175	1	7.5	15.0
Amosite + PWG[b]	100	100	1	–	–

[a]Gavage with 1,2-dimethylhydrazine dihydrochloride.
[b]Animal was inadvertently gavaged during preweaning with intermediate (medium)-range chrysotile instead of amosite.

Clinical Signs

The incidence of clinical signs occurred at essentially comparable frequencies throughout the study groups except those that received DMH (see below). No distinct signs of compound effect were noted in any of the tremolite- or amosite-treated animals during the first 52 weeks of study. As the study proceeded, the incidence of clinical signs increased among all the groups. At intervals where there were a large number of moribund sacrificed animals in any one particular group, the clinical signs most frequently observed were supportive of the conditions for moribund sacrifice previously outlined in the Methods section. A comparison of clinical signs observed during the same selected intervals among all the groups revealed a larger number of palpable abdominal masses, tissue masses, and central nervous system signs, a well as red discharge and protruding masses from the rectum in the DMH and amosite and DMH groups. These findings were presumably due to the administration of DMH since they were not clinically observed with any frequency in any of the tremolite- or amosite-treated groups.

Body Weight and Food Consumption

Mean body weights were analyzed at selected intervals: birth, 3, 8, 11, 15, 24, 33, and 60 weeks for the males, and birth, 3, 8, 11, 16, 27, 48 and 60 weeks for the females by the method of Rao (*9*). The data revealed a 13% depressed mean body weight gain at weaning in both sexes of the tremolite groups and 15% in the amosite groups compared to the controls. The depressed weight gain in the tremolite- and amosite-exposed rats was more apparent at 8 weeks of age (tremolite:33% for males and 17% for females; amosite: 37% for males and 25% for females). Weight gain then paralleled the controls (except for DMH-exposed rats) for the remainder of the study but the mineral-exposed rats remained smaller throughout the study. Both male and female DMH-exposed groups gained less than their respective controls.

In the tremolite-exposed males and females, the average weekly food consumption was 97% that of the untreated controls. In the DMH, amosite, amosite and DMH, and amosite and PWG males, the mean weekly food consumption was 102%, 102%, 105%, and 107%, respectively, compared to the untreated control group and 98%, 101%, 105%, and 108% that of the untreated control for comparable groups of females.

Survival

Survival data of intervals prior to the final sacrifice of a group are summarized in Table 7. There were no significant differences in survival between the tremolite-exposed and control groups. Survival of males and females was approximately equal until 112 weeks, after which the females tended to live longer. When compared to the survival rates of the untreated control group, the amosite male survival at 118 weeks was higher, while amosite and PWG male survival was somewhat less. In female rats, the amosite group survival was better than the untreated controls, while the amosite and PWG group was about the same. The survival of both groups of DMH-exposed rats was considerably less than the untreated controls. The amosite plus DMH group was comparable to the DMH alone group.

Pathology

There were no apparent treatment related neoplasms in the digestive tract of the tremolite, amosite, or amosite PWG groups (Tables 8 and 9). Also, no specific type was increased, either at a particular location (e.g., cecum) or in the stomach, small or large intestine as a whole. In addition, the incidences of non-neoplastic diseases of the gastrointestinal tract such as enteritis, diverticulitis, ulceration or inflammation in general were comparable in the control and tremolite- or amosite-exposed rate (Tables 10 and 11).

There were no organs/tissues in the tremolite-exposed rats which showed an increased rate of neoplasia compared to the control groups. Organs which showed an increase in neoplasms in the amosite or amosite PWG groups compared to the control group were the thyroid and hematopoeitic system. The results are as follows.

Thyroid. Table 12 summarizes the incidence

Table 7. Survival of F344 rats in lifetime oral asbestos study at various time points.

Group	Age, weeks	Males No. alive/ total no.	Males Survival, %	Females No. alive/ total no.	Females Survival, %
Control	106	98/118	83	97/118	82
	120	70/118	59	71/118	60
	146	6/118	5	20/118	17
	148	–	–	14/118	12
Tremolite	106	206/250	82	207/247	84
	120	150/250	60	144/247	58
	146	36/250	14	33/246	13
	148	–	–	22/246	9
Control	106	95/117	81	92/117	79
	118	71/117	61	62/117	53
	142	7/117	6	20/116	17
	146	–	–	10/116	9
DMH	106	27/125	22	15/125	12
	118	16/125	13	–	–
	142	–	–	–	–
	146	–	–	–	–
1% Amosite	106	221/250	88	202/246	82
	118	117/250	71	162/246	66
	142	35/249	14	43/245	18
	146	–	–	28/245	11
1% Amosite + DMH	106	46/175	26	32/174	18
	118	26/175	15	–	–
	142	–	–	–	–
	146	–	–	–	–
1% Amosite + PWG	106	77/100	77	86/100	86
	118	52/100	52	56/100	56
	142	6/100	6	15/100	15
	146	–	–	9/100	9

of thyroid C-cell proliferative lesions. A significantly increased incidence of C-cell carcinoma was found in amosite-treated male rats ($p < 0.05$). This effect was not observed in amosite PWG male rats. Furthermore, the overall incidence of C-cell tumors (adenomas and/or carcinomas) was comparable between control and treated groups. C-cell hyperplasia was equivocally increased in amosite and amosite PWG female groups.

Hematopoietic System. A significantly increased incidence of mononuclear cell leukemia occurred in amosite ($p < 0.05$) and amosite PWG ($p < 0.01$) male rats (Table 13). However, neither group was significant when compared to the tremolite control group (39%). This increased incidence was not observed in amosite-exposed females.

Miscellaneous Neoplasms

Occasionally a somewhat higher or lower rate of commonly occurring neoplasms were observed in amosite treated groups. A statistically significant ($p < 0.05$) decrease in the rate of neoplasia was observed in the pancreas (Islet cell adenoma), adrenal medulla (pheochromacytoma), thyroid (follicular cell carcinoma) and preputial gland in at least one group of amosite-exposed rats compared to the controls. Similar observations were not observed in the tremolite-exposed groups.

Nonneoplastic Findings

A plethora of incidental lesions of aging Fischer 344 rats was found in all groups. Statistical analyses showed no obvious correlation between the incidence of specific lesion types and the type of treatment. Nonneoplastic lesions that were observed in more than 5% of the rats in any of the experimental groups are as follows: skin: epidermal inclusion cyst; lung: chronic inflammation (peribronchiolar and perivascular lymphoid cuffing); spleen: fibrosis, hemosiderosis, extramedullary hematopoeisis, lymphoid atrophy; lymph

Table 8. Number of tremolite-exposed F344 rats with primary epithelial neoplasms of the alimentary tract.

	Males[a]		Females[a]	
	Control	Tremolite	Control	Tremolite
Animals examined	118	250	118	250
Total alimentary	8(7)	12(5)	3(3)	7(3)
Oral/pharynx				
Papilloma	1(1)	1(0)	0(0)	1(0)
Carcinoma	3(3)	1(0)	2(2)	5(2)
Esophagus				
Total gastrointestinal	4(3)	9(3)	1(1)	1(0)
Total stomach	3(3)	2(1)	0(0)	0(0)
Nonglandular				
Papilloma	2(2)	1(0)		
Carcinoma	1(1)	1(0)		
Glandular				
Polyp				
Carcinoma				
Total small intestine	0(0)	3(1)	0(0)	0(0)
Polyp		1(0)	1(1)	
Adca in polyp[b]				
Carcinoma		2(1)		
Total large intestine	1(1)	4(2)	0(0)	0(0)
Cecum				
Polyp		1(0)		
Adca in polyp[b]				
Carcinoma				
Colon				
Polyp	1(1)	1(0)		
Adca in polyp[b]				
Carcinoma		2(1)		

[a]Values in parentheses are percentages.
[b]Adenocarcinoma arising in adenomatous polyp.

nodes (various): lymphoid or reticulum cell hyperplasia, lymph-angiectasis, hemorrhage, pigmentation, chronic inflammation; heart: chronic inflammation; liver: degeneration, necrosis, fatty metamorphosis, toxic hepatitis (associated with leukemia), granuloma, angiectasis, pigmentation, focal cellular change; bile duct (extrahepatic): chronic inflammation, mucosal hyperplasia, cysts, fibrosis; pancreas (exocrine): atrophy, hyperplasia, ectopia; pancreas (endocrine): hyperplasia; kidney: chronic progressive nephropathy, cysts, pigmentation; pituitary gland: cysts, angiectasis, hyperplasia; adrenal (cortex): fatty metamorphosis, hyperplasia; adrenal (medulla): hyperplasia; thyroid: follicular cysts, C-cell hyperplasia; parathyroid: hyperplasia; testes: seminiferous degeneration, interstitial cell hyperplasia; prostate: abscess, chronic inflammation, glandular hyperplasia; seminal vesicles: cysts; ovary: follicular and parovarian cysts; uterus: hydrometra, endometrial cyst; mammary gland: cystic ducts, glandular hyperplasia, galactocele; mesentery: chronic inflammation; eye: cataract, hemorrhage, inflammation, retinal degeneration: zymbal gland: cystic ducts; bone: osteopetrosis, exostoses, marrow hyperplasia. Alimentary tract nonneoplastic lesions are noted in Tables 10 and 11.

1, 2-Dimethylhydrazine Dihydrochloride-Treated Groups

Two groups of male and female rats were exposed to 1, 2-dimethylhydrazine dihydrochloride (DMH) by gavage at levels of 7.5 mg/kg for males and 15.0 mg/kg for females, biweekly for a total of five doses. One group served as a positive carcinogen control and the other received amosite from weaning throughout life.

Exposure of rats to DMH or DMH with amosite was associated with a dramatically increased incidence of neoplasms of the intestinal tract, Zymbal's gland, and liver of male and female rats, and kidney in female rats. It is also noteworthy that survival in the DMH groups was shortened due to the presence of these neoplasms.

Table 9 summarizes the numbers of rats with primary epithelial neoplasms in the gastrointestinal tract by specific site and classification. Intestinal neoplasms, particularly the adenomatous polyps, were often multiple within a given animal.

Table 9. Number of amosite-exposed F344 rats with primary epithelial neoplasms of the gastrointestinal tract.

	Untreated control[a]		Amosite[a]		Amosite PWG[a]	
	M	F	M	F	M	F
Animals examined	117	117	249	250	100	100
Total gastrointestinal	4(4)	2(2)	7(3)	4(2)	3(3)	3(3)
Total stomach	1(1)	1(1)	2(1)	1(0)	0(0)	0(0)
Total small intestine	3(3)	0(0)	2(1)	3(1)	1(1)	1(1)
Duodenum						
Carcinoma			1(0)		1(1)	
Adca in polyp[b]						
Adenomatous polyp						
Jejunum						
Carcinoma	2(2)			1(0)		1(1)
Adca in polyp[b]			1(0)			
Adenomatous polyp				2(1)		
Ileum						
Carcinoma						
Adca in polyp[b]						
Adenomatous polyp	1(1)					
Total large intestine	0(0)	1(1)	3(1)	0(0)	2(2)	2(2)
Cecum						
Carcinoma						
Adca in polyp[b]						
Adenomatous polyp					1(1)	
Total colon	0(0)	1(1)	3(1)	0(0)	1(1)	2(2)
Ascending colon						
Carcinoma			1(0)			
Adca in polyp[b]						
Adenomatous polyp						
Transverse colon						
Carcinoma						1(1)
Adca in polyp[b]						
Adenomatous polyp						
Descending colon						
Carcinoma						
Adca in polyp[b]					1(1)	
Adenomatous polyp		1(1)	2(1)			1(1)

[a]Values in parentheses are percentages.
[b]Adenocarcinoma arising in adenomatous polyp.

The incidence of gastrointestinal neoplasia was dramatically increased with DMH treatment. However, the incidence appeared to be essentially comparable between groups receiving DMH alone and those receiving DMH with amosite. Furthermore, the number of animals with tumors either in the small intestine or in the large intestine was also essentially comparable between DMH alone and DMH with amosite. There was no difference in the time to tumor between the groups.

Evaluation of the incidence of the three categories of intestinal neoplasia (carcinoma, adenocarcinoma arising in an adenomatous polyp, and adenomatous polyp) by site (Table 14) reveals an increased incidence of duodenal carcinoma ($p < 0.05$) in the DMH with amosite-treated females, compared to female rats receiving DMH alone. In the jejunum, however, this incidence is reversed, with more carcinomas occurring in the female group receiving DMH alone.

In the large intestine the frequency of carcinoma arising in an adenomatous polyp and adenomatous polyps was greatest in the descending colon. In the cecum, the incidence of carcinoma was less in the DMH with amosite-treated group than those treated with DMH alone, in male rats. This effect was not observed in the female group. The appearance of carcinomas in the ascending colon was somewhat greater in DMH with amosite-treated males than in males receiving DMH alone. Adenocarcinoma arising in adenomatous polyp occurred more frequently in the transverse colon of male and female rats receiving DMH with amosite compared to rats receiving DMH alone.

Kidney Neoplasms

Almost without exception, the renal masses associated with DMH treatment were malignant

Table 10. Incidence of nonneoplastic lesions in the alimentary tract of F344 rats exposed to 1% tremolite in the diet.[a]

	Males[b]		Females[b]	
	Control	Tremolite	Control	Tremolite
Animals examined	118	250	118	250
Palate/tongue				
Inflammation	0(0)	0(0)	0(0)	4(2)
Necrosis	0(0)	0(0)	0(0)	1(0)
Hyperkeratosis	0(0)	1(0)	0(0)	2(1)
Acanthosis	1(1)	3(1)	1(1)	1(0)
Esophagus				
Inflammation	1(1)	0(0)	0(0)	1(0)
Necrosis	2(2)	1(0)	0(0)	0(0)
Hyperkeratosis	9(8)	18(7)	3(3)	4(2)
Acanthosis	1(1)	0(0)	0(0)	1(0)
Stomach-nonglandular				
Mineralization	13(11)	5(2)	4(3)	2(1)
Inflammation, chronic	19(16)	29(12)	25(21)	38(15)
Ulceration	10(8)	17(7)	9(8)	11(4)
Necrosis	20(17)	46(18)	17(4)	31(12)
Hyperplasia	3(3)	1(0)	0(0)	2(1)
Hyperkeratosis	18(15)	34(14)	15(13)	29(12)
Acanthosis	26(22)	54(22)	23(19)	45(18)
Stomach-glandular				
Hyperplasia	7(6)	1(0)	3(3)	0(0)
Small intestine				
Inflammation	0(0)	2(1)	0(0)	1(0)
Necrosis	2(2)	0(0)	1(1)	3(1)
Ulceration	0(0)	1(0)	0(0)	0(0)
Colon				
Parasitism	5(4)	32(13)	5(4)	3(1)
Inflammation	0(0)	5(2)	3(3)	0(0)
Necrosis	0(0)	3(1)	1(1)	1(0)
Hyperplasia	0(0)	1(0)	0(0)	1(0)
Cecum				
Parasitism	9(8)	2(1)	2(2)	1(0)
Inflammation	1(1)	2(1)	4(4)	1(0)
Necrosis	1(1)	4(2)	1(1)	3(1)
Hyperplasia	0(0)	0(0)	0(0)	1(0)
Rectum				
Necrosis	0(0)	1(0)	0(0)	0(0)
Anus (no lesions)				

[a]Incidence of nonneoplastic lesions that occur with a frequency of 1% or more in at least one group.
[b]Values in parentheses are percentages.

mesenchymal or mixed malignant tumors. Purely mesenchymal growths were classified according to their morphology (i. e., fibrosarcoma, undifferentiated sarcoma). Those having epithelial elements or epithelial-like elements were classified as mixed malignant tumors. In early stages, these neoplasms appeared as interstitial sclerosing growths near the inner cortex. Collagen formation was accompanied by proliferating, basophilic, primitive-appearing cells. Epithelial elements consisted of glands, ductlike structures or poorly differentiated solid tubules. The growths were often massive but rarely metastasized.

Table 15 summarizes the incidence of kidney tumors in control and DMH-treated groups. The high incidence of renal neoplasms was confined almost exclusively to treated female rats receiving either DMH alone or DMH with amosite ($p < 0.01$). The incidence rates for the two treated female groups was the same. Renal neoplasms occurred infrequently in male rats.

Zymbal Gland Neoplasms

Carcinoma was the most commonly observed neoplasm in Zymbal's gland. These neoplasms were composed of proliferating eosinophilic to basophilic squamous epithelial cells which formed thick fingers of tissue, masses of keratin and nests of sequestered cells. Some had sebaceous features with formation of sebum. Infiltration of adjacent tissues was not uncommon; however, metastases were rare. Table 16 summarizes the number of control and DMH-treated rats with Zymbal's gland neoplasms.

Table 11. Incidence of nonneoplastic lesions in the alimentary tract of F344 rats exposed to amosite asbestos.[a]

	Control[b]		Amosite[b]		Amosite PWG[b,c]	
	M	F	M	F	M	F
Tongue, number examined	117	117	249	250	100	100
Esophagus, number examined	115	117	249	246	100	100
Hyperkeratosis	12(10)	7(6)	4(2)	7(3)	12(12)	6(6)
Stomach, nonglandular, number examined	117	117	249	250	100	100
Mineralization	9(8)	3(3)	2(1)	2(1)	1(1)	0(0)
Inflammation, chronic	21(18)	21(18)	56(22)	60(24)	17(17)	18(18)
Ulceration	13(11)	4(3)	25(10)	30(12)	7(7)	10(10)
Necrosis	23(20)	13(11)	41(16)	37(15)	15(15)	11(11)
Hyperkeratosis	22(19)	24(21)	41(16)	56(22)	16(16)	17(17)
Acanthosis	31(26)	26(22)	62(25)	72(29)	21(21)	23(23)
Muscle degeneration	8(7)	2(2)	3(1)	3(1)	0(0)	0(0)
Stomach, glandular, number examined	117	117	249	250	100	100
Hyperplasia	6(5)	2(2)	0(0)	1(0)	0(0)	0(0)
Duodenum, number examined	117	117	249	249	100	100
Jejunum, number examined	117	117	249	249	100	100
Ileum, number examined	117	117	249	249	100	100
Colon, number examined	117	117	249	250	100	100
Parasitism	4(3)	2(2)	17(7)	6(2)	4(4)	8(8)
Cecum, number examined	117	117	249	250	100	100
Rectum, number examined	117	117	249	250	100	100
Anus, number examined	117	117	249	250	100	100

[a]Incidence of nonneoplastic lesions that occur with a frequency of 1% or more in at least one group.
[b]Values in parentheses are percentages.
[c]PWG = preweaning gavage.

Table 12. Number of F344 rats with thyroid C-cell proliferative lesions.

	Control[a]		Amosite[a]		Amosite PWG[a]	
	M	F	M	F	M	F
Animals examined	117	116	246	247	100	100
Total C-cell tumors	27(23)	24(21)	76(31)	65(26)	25(25)	29(29)
C-cell adenoma	16(14)	14(12)	26(11)	37(15)	11(11)	15(15)
C-cell carcinoma	11(9)	10(9)	50*(20)	29(12)	14(14)	14(14
C-cell hyperplasia	21(18)	22(19)	58(24)	71(29)	23(23)	26(26)

[a]Values in parentheses are percentages.
*$p < 0.05$ vs. controls (incidental tumor and Fisher exact tests).

Table 13. Number of amosite-exposed F344 rats with mononuclear leukemia.

	Untreated control		Amosite		Amosite PWG	
	M	F	M	F	M	F
Animals examined	117	117	249	250	100	100
Mononuclear cell leukemia[a]	38(32)	40(34)	106*(42)	82(33)	49†(49)	34(34)

[a]Values in parentheses are percentages.
*$p < 0.05$ vs. controls (incidental tumor and Fisher's exact test).
†$p < 0.01$ vs. controls.

Approximately one quarter of all rats receiving DMH alone or DMH with amosite developed Zymbal's gland neoplasms ($p < 0.01$), while in control animals the occurrence was low (1–3%). The incidence appeared essentially comparable between the two DMH-treated groups.

Liver Neoplasms

The classification of hepatocellular proliferative lesions was based on the ILAR Monograph (*10*). Table 17 summarizes the number of control or DMH-treated rats with neoplastic nodules or hepatocellular carcinoma.

Table 14. Number of DMH-exposed F344 rats with primary epithelial neoplasms of the gastrointestinal tract.

	Untreated control[a]		DMH positive control[a]		DMH with amosite[a]	
	M	F	M	F	M	F
Animals examined	117	117	125	124	173	175
Total gastrointestinal	4(4)	2(2)	92(74)	77(62)	118(68)	114(65)
Total stomach	1(1)	1(1)	3(2)	4(3)	2(1)	1(1)
Total small intestine	3(3)	0(0)	18(14)	14(11)	19(11)	24(14)
Duodenum						
Carcinoma	0(0)	0(0)	11(9)	3(2)	13(8)	19(11)
Adca in polyp[b]						
Adenomatous polyp						
Jejunum						
Carcinoma	2(2)	0(0)	2(1)	11(9)	3(2)	2(1)
Adca in polyp[b]					1(1)	
Adenomatous polyp			1(1)			
Ileum						
Carcinoma			2(1)			
Adca in polyp[b]						1(1)
Adenomatous polyp	1(1)					
Total large intestine	0(0)	1(1)	81(65)	70(56)	110(64)	101(58)
Cecum						
Carcinoma			16(13)	7(6)	6(3)	6(3)
Adca in polyp[b]						
Adenomatous polyp					2(1)	
Total colon						
Ascending colon						
Carcinoma			10(8)	10(8)	20(12)	14(8)
Adca in polyp[b]			2(1)	4(3)	3(2)	7(4)
Adenomatous polyp			5(4)	7(6)	5(3)	8(5)
Transverse colon						
Carcinoma			1(1)		2(1)	2(1)
Adca in polyp[b]			8(6)	9(7)	21(12)	20(11)
Adenomatous polyp	8(6)	6(5)	22(13)	22(13)		
Descending colon						
Carcinoma				(1)	3(2)	2(1)
Adca in polyp[b]			20(16)	15(12)	25(14)	22(13)
Adenomatous polyp		1(1)	34(27)	27(22)	40(23)	31(18)
Colon (other)[c]						
Carcinoma				4(3)	2(1)	2(1)
Adca in polyp[b]						
Adenomatous polyp			1(1)	1(1)		1(1)

[a]Values in parentheses are percentages.
[b]Adenocarcinoma arising in adenomatous polyp.
[c]Colon (other) = site not identified.

Table 15. Number of DMH-exposed F344 rats with primary renal neoplasms.

	Untreated control		DMH positive control		DMH with amosite	
	M	F	M	F	M	F
Animals examined	117	117	125	124	173	175
Total renal tumors[a]	0(0)	1(1)	3(2)	49(32)*	4(2)	56(32)*

[a]Values in parentheses are percentages.
*$p = < 0.01$.

A significantly increased incidence of neoplastic nodules and/or hepatocellular carcinomas occurred in groups receiving DMH alone and in groups receiving DMH plus amosite. Generally, females had a higher incidence ($p < 0.01$) than males ($p < 0.05$).

Miscellaneous Neoplasms

In several instances, DMH treatment with or without amosite led to statistically significant decreased incidences of certain spontaneous neoplasms, particularly of the endocrine system.

Table 16. Number of DMH-exposed F344 rats with Zymbal gland neoplasms.

	Untreated control		DMH positive control		DMH with amosite	
	M	F	M	F	M	F
Animals examined	117	117	125	124	173	175
Zymbal gland neoplasms[a]	1(1)	4(3)	33(26)*	34(27)*	55(32)*	39(22)*

[a]Values in parentheses are percentages.
*$p < 0.01$ vs. controls.

Table 17. Number of DMH-exposed F344 rats with hepatocellular neoplasms.

	Untreated control		DMH positive control		DMH with amosite	
	M	F	M	F	M	F
Animals examined	117	117	125	124	173	175
Neoplastic nodules[a]	9(8)	4(3)	18(14)*	29(23)*	27(15)*	32(18)*
Hepatocellular carcinoma[a]	1(1)	1(1)	9(7)*	10(8)*	7(4)†	8(5)‡

[a]Values in parentheses are percentages.
*$p < 0.05$ vs. controls.
†$p < 0.05$ vs. controls (incidental tumor and life table analysis).
‡$p < 0.05$ vs. controls (life table analysis only).

These included a reduced number of subcutaneous fibromas, pituitary adenomas in females, adrenal pheochromocytomas, pancreatic acinar cell adenomas and islet cell adenoma in males, mammary tumors, and interstitial cell tumors in male rats. However, many animals in these two groups died at an early age compared to the untreated controls.

Discussion

Tremolite (*11*) or amosite asbestos (*12*) was administered at a level of 1% in the diet to male and female F344 rats for their lifetime, including exposure of their dams to the test material. While the tremolite used in this study is considered crystalline or nonfibrous in its natural form, a small amount assumes a fibrous character during the crushing and milling process. However, the milling process used in the preparation of the tremolite for this study was identical to what is done in the commercial setting.

Starting at birth, one of three groups of neonate rats from amosite-exposed mothers were given chrysotile asbestos (instead of amosite) by gavage until weaning at which time they were given the 1% amosite diet. For all intents and purposes this group of rats should be regarded as being exposed to amosite asbestos for their lifetime. Two groups (control and amosite exposed) of weanling rats were exposed to five biweekly doses of 1,2-dimethylhydrazine dihydrochloride (DMH), a known intestinal carcinogen, to test the promotor or cocarcinogenic effects of DMH and amosite asbestos.

The clinicopathologic results in this study showed that the ingestion of tremolite or amosite asbestos did not adversely affect the fertility of the mothers or litter size of the F_1 bioassay animals. The average weight of the offspring at birth from mothers exposed to either mineral was comparable to the offspring of nonexposed mothers. However, the weight of the exposed offspring a weaning was slightly less than the control rats. The cause of the decreases in weight during lactation is not known. The differences in body weight gain became more apparant between weaning and 8 weeks of age. While the tremolite- and amosite asbestos-exposed rats paralleled the control animals in weight gain, they remained smaller throughout their life. The mean body weight of the male rats exposed to the chrysotile preweaning gavage (PWG) and subsequently to amosite asbestos was slightly higher than the amosite alone rats. This may be related to the mortality induced in the neonates by the PWG technique which would allow the remaining pups more milk during lactation. Exposure to DMH caused a small reduction in body weight gain in female but not in male rats.

No clinical signs were observed which could be attributed to the ingestion of either mineral. Starting at 9 months of age, the DMH-exposed rats showed signs attributable to DMH-related neoplasia, but no difference was noted between the DMH and DMH plus amosite groups.

The ingestion of either tremolite or amosite in the diet for the life of the rats did not adversely affect their survival. In fact, survival of female rats exposed to amosite or amosite plus chrysotile PWG was slightly better up to 112 weeks than the controls. Similarly, the survival of male rats exposed to amosite was slightly better than the untreated controls, although the amosite plus chrysotile PWG group showed slightly less survivability.

The most plausible explanation for the increased survival of the amosite exposed rats is their lower weight throughout the study. Yu et al. (*13*) have shown that rats of lower body weight caused by restricted caloric intake lived longer than rats that were allowed to eat an unlimited amount of food.

The survival of the rats (control and amosite) in this study compares favorably with other NTP bioassays (*14*). At 106 weeks of age (age at end of typical 2-yr bioassay) the percentage of male rats alive in this study was: untreated tremolite control, 83%; untreated amosite control, 81%; tremolite, 82%; amosite, 88%; and amosite plus PWG, 77%. The percentage of female rats alive at this time was: tremolite control, 82%; amosite control, 79%; tremolite, 84%; amosite, 82%; and amosite plus PWG, 86%. Haseman (*14*) in reviewing the 25 most recent NTP feeding studies found an average of 66% of control males and 73% of control females alive at 112 weeks of age.

The survival of control groups of males and females was similar at 106 weeks. In most 2-yr studies involving rats, more females generally survive to the end of the study than do males. However, the longer survival of female rats (control and tremolite or amosite exposed) was clearly demonstrated after 142 weeks.

The ingestion of either tremolite or amosite asbestos over the lifetime of these rats did not cause a biologically significant increase of neoplasms at any anatomic site when compared to the concurrent controls. The gastrointestinal tract was considered a potential target organ based on epidemiological studies in humans (*15*). The overall incidence of intestinal neoplasms in the control (male 4 and female 2%) and two amosite asbestos groups (male 3 and female 2%; male 4 and female 3%) was low, and there was no significant ($p < 0.05$) difference between the treated and control groups. Similar observations were noted in the tremolite groups and their respective controls. In addition, nonneoplastic lesions of the gastrointestinal tract were not increased. In summary, amosite asbestos did not cause any adverse affects in the gastrointestinal tract of either male or female F344 rats.

Rats exposed to DMH showed a high incidence (60–70%) of neoplasia of the gastrointestinal tract, primarily in the large intestine. This high rate of intestinal neoplasia was unexpected because a pilot study (*3*) using the same dosing regimen of DMH would have predicted an incidence of 15 ± 5% in this study. In a previous NTP bioassay, hamsters exposed to DMH and chrysotile asbestos also failed to develop the desired rate of intestinal tumors based on a similar pilot study (*28*). Apparently the neoplastic dose response to DMH is relatively steep and duplication of low rates of intestinal neoplasia are difficult to reproduce.

Because of the high background rate of DMH-induced neoplasia, it is not possible to determine with accuracy if amosite had a cocarcinogenic or additive effect in this study. Female rats exposed to DMH and amosite had a higher incidence (11% versus 2%) of neoplasia of the duodenum than the DMH controls. Conversely, they had a lower incidence (9% versus 1%) of neoplasms of the jejunum; thus the total number of animals with neoplasms of the small intestine was comparable. A similar situation was observed in the large intestine of male rats. The rats exposed to DMH alone had a higher incidence (13% versus 3%) of carcinoma of the cecum but a lower incidence (13% versus 26%) of neoplasms of the transverse colon.

The morphologic appearance of the neoplasms induced by DMH were comparable to those described previously in rats exposed to hydrazine compounds (*16*). In addition, the few intestinal neoplasms which occurred in the control and tremolite- or amosite (no DMH)-exposed rats were of the same morphologic types to those induced by DMH. The neoplasms observed in the kidney, liver and Zymbal's gland of DMH-exposed rats were consistent with those reported for these types of intestinal carcinogens (*17*).

A significantly ($p < 0.05$) increased incidence of C-cell carcinomas of the thyroid occurred in amosite-treated male rats. This effect was not observed in the amosite PWG male rats and the overall incidence of total benign and malignant C-cell tumors was comparable between control and treated groups. Therefore, this is not considered to be a treatment-related effect.

The incidence of mononuclear cell leukemia (synonyms—monocytic leukemia, Fisher rat leukemia) was elevated in amosite (42%) and amosite PWG (49%) male rats compared to the concurrent control group (32%). However, the rates were not significant when compared to the tremolite male control group (39%). This increased inci-

dence was not observed in treated female rats. Coleman et al. (*18*) reported an incidence of nearly 30% in male F344 rats within the age group of 24–40 months. In 2-yr-old F344 rats, Goodman et al. (*19*) reported 12% of males and nearly 10% of females had lymphoma/leukemia, a much lower incidence than in these studies. It is apparent from this study and above cited studies that the incidence of leukemia increases rapidly after 2 years of age. In view of considerable variation in the incidence of such disorders, the fact that the amosite-exposed male rats survived longer than their concurrent controls and lack of significance when compared to the tremolite control group, it is doubtful that the increase in the rate of leukemia is treatment related. More importantly, an increased incidence of neoplasia was not observed in target organs (GI tract and mesothelium). Even though it is known that certain types of asbestos are absorbed through the GI tract (*20,21*), it is difficult to envision how oral asbestos could cause an increase in leukemia without causing an increase of tumors in the proposed target tissues.

In summary, these effects represent only a modulation of neoplasms which occur in concurrent control groups and are known to occur in historical control rats of this strain. No uncommon or unique neoplasms were observed in any of the tremolite- or amosite-treated groups. In addition, the biological importance of the neoplasms in the absence of target organ neoplasia is questionable.

A large variety of nonneoplastic lesions, primarily lesions of aging, were observed in all groups. There was no obvious correlation between treatment and specific lesions. Therefore, tremolite or amosite at the level of 1% in the diet did not appear to cause any overt toxicity.

Studies on the effects of chronic ingestion of tremolite are not available. However, Stanton et al. (*2*) showed that the intrapleural inoculation of fibrous tremolite (two types) caused a high incidence of pleural sarcoma in Osborne-Mendel rats. In contrast, intrapleural studies of tremolite talc failed to show a carcinogenic response in hamsters (*22*). The tremolite used in the NTP study is a nonfibrous type and more closely resembles that used by Smith (*22*) than Stanton et al. (*2*).

Other studies involving the long-term ingestion of asbestos are few. Donham et al. (*23*) reported equivocal results in F344 rats which were fed a diet containing 10% chrysotile for their lifetime. While they did not observe a statistically significant ($p < 0.05$) increase in the number of tumors in exposed animals, the authors believed that there was a trend toward increased colon lesions in general, evidence of penetration of asbestos into the colonic mucosa, possible cytotoxicity to colonic tissues and suggested a possible relationship to peritoneal mesothelioma. Another equivocal study is that reported by Gibel et al. (*24*), who described an increase in malignant tumors in the lung, kidney, liver and reticuloendothelial system but no increase in intestinal neoplasia in Wistar rats fed asbestos filter material (20 mg/day) for a period of 8–14 months. Cunningham et al. (*25*) reported two studies in Wistar male rats using 1% chrysotile in the diet: one study of 24 months and one of 30 months. These authors concluded that trace amounts of ingested asbestos can penetrate the walls of the gastrointestinal tract, but evidence of carcinogenicity was inconclusive. Negative results were reported by Gross et al. (*26*), who fed rats a diet containing 5% chrysotile asbestos for a period of 21 months with no evidence of intestinal neoplasia.

Corollary studies to this investigation were conducted in Syrian golden hamsters (*27, 28*). The exposure regimen was similar in that male and female hamsters were exposed to 1% amosite asbestos (same source as the subject study) and short-range or intermediate-range chrysotile asbestos in their diet for their natural life-span. There was no adverse effect on body weight gain or survival, and no asbestos-related neoplasms were observed.

Another oral asbestos study in hamsters was reported by Smith et al. (*29*). They exposed groups of 30 male and female hamsters via drinking water for lifetime to amosite asbestos, mine tailings, beach rock or Lake Superior drinking water. They did not observe adverse effects on body weight or survival time in any of the groups. A peritoneal mesothelioma, one pulmonary carcinoma, and two early squamous cell carcinomas of the nonglandular stomach were found in the hamsters exposed to amosite but the incidence was not statistically significant ($p < 0.05$). They concluded that the study was essentially negative. A subsequent study in rats using similar materials also failed to elicit a carcinogenic response (*30*).

Except for the studies of Donham et al. (*23*), Smith et al. (*29*) and the NTP (*11, 12, 27, 28*), the other studies were conducted with relatively small numbers of animals. Also some were conducted for an insufficient period of time to adequately test the carcinogenic potential of ingested asbestos.

A long-term study of amosite asbestos designed to determine the promotor potential of asbestos

was reported by Ward et al. (*31*). They exposed 6-week-old male F344 rats three times per week for 10 weeks to 1 mg amosite asbestos in saline via gavage. Once per week during this same period, half of the rats received subcutaneous injections of 7.4 mg/kg azoxymethane (AOM), a known intestinal carcinogen in animals which produces effects similar to DMH. The rats were allowed to live out their lifespan or untl 94–95 weeks of age at which time they were killed. The authors reported an intestinal tumor incidence of 66.7% in AOM alone, 77.1% for amosite plus AOM and 32.6% for amosite alone. The authors concluded that while amosite did not significantly add to the incidence of AOM-induced intestinal neoplasia, amosite alone caused a relatively high rate of intestinal neoplasia. However, there was no untreated control group to compare to the treated groups. These results should also be viewed with some suspicion because the authors also reported a 14% incidence of Zymbal gland tumors in the rats exposed to amosite alone. The historical rate of Zymbal gland tumors in the Bioassay Program is 0.34%, indicating that this is a relatively rare tumor (*19*). However, AOM is known to induce Zymbal gland tumors with a sngle dose of 5.1 mg/kg in male F344 rats producing a 14% incidence of tumors in this organ (*17*); in this study 5.1 mg/kg AOM also caused a 24% incidence of intestinal neoplasia. A possible explanation for the incidence of Zymbal gland tumors in the amosite groups would be that they were inadvertently exposed to AOM. If this occurred, these rats would also be expected to show a high incidence of intestinal neoplasms.

Conclusions

Under the conditions of this lifetime bioassay, tremolite or amosite asbestos was not toxic, did not affect survival, and was not carcinogenic when ingested at a level of 1% in the diet by male and female Fischer 344 rats. While there were significant ($p > 0.05$) increases in the rate of C-cell carcinomas of the thyroid in male, and monocytic (mononuclear cell) leukemia in male rats exposed to amosite asbestos compared to untreated controls, their biological significance is questionable because of a lack of response in the concurrent amosite and preweaning gavage group or control group of the corollary study, nonaffect when all neoplasms of that organ are analyzed, lack of significance when examined using life table analysis or the absence of neoplasia in target organs. The cocarcinogenic studies using 1,2-dimethylhydrazine dihydrochloride (DMH) were considered flawed because of the high rate of intestinal carcinogenesis in both the DMH and amosite asbestos and DMH alone groups.

The animal phase of this study was performed at Hazleton Laboratories America, Vienna, VA. This project has been funded with federal funds from the National Institute of Environmental Health Sciences (NIH), Research Triangle Park, NC, under contract #5-2158. Partial funding was provided by the Environmental Protection Agency under Interagency Agreement #D70756.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Proceedings of the Joint NIEHS-EPA Conference on Biological Effects of Ingested Asbestos. Environ. Health Perspect. 9: 113–462 (1974).
2. Stanton, M. F., Layard, M., Tegeris, A., Miller, E., May, M., Morgan, E., and Smith, A. Relation of particle dimension to the carcinogenicity in amphibole asbestoses and other fibrous minerals. J. Natl. Cancer Inst. 67: 965–975 (1981).
3. McConnell, E. E., Wilson, R. E., Moore, J. A., and Haseman, J. K. Dose response of 1,2-dimethylhydrazine and methylazoxymethanol acetate in the F344 rat. Cancer Letters 8: 271–278 (1980).
4. Kaplan, E. L., and Meier, P. Nonparametric estimation from incomplete observations. J. Am. Statist. Assoc. 53: 457–481 (1958).
5. Cox, D. R. Regression models and life tables. J. Roy. Statist. Soc. B34: 187–220 (1972).
6. Mantel, N., and Haenszel, W. Statistical aspects of the analysis of data from retrospective studies of disease. J. Natl. Cancer Inst. 22: 719–748 (1959).
7. Peto, R., Pike, M., Day, N., Gray, R., Lee, P., Parish, S., Peto, J., Richard, S., and Wahrendorf, J. Guidelines for simple, sensitive significant tests for carcinogenic effects in long-term animal experiments. IARC. Monographs on the Long-Term and Short-Term Screening Assays for Carcinogens: A Critical Appraisal. World Health Organization, Geneva; Supplement 2, 1980, pp. 311–426.
8. Gart, J., Chu, K., and Tarone, R. Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. J. Natl. Cancer Inst. 62: 957–974 (1979).
9. Rao, C. R. Some statistical methods for comparison of growth curves. Biometrics 14: 1–17 (1958).
10. Stewart, H. L. (Ed.). ILAR monograph: Histologic typing of liver tumors of the rat. J. Natl. Cancer Inst. 64: 179–206 (1980).
11. NTP. Carcinogenesis bioassay of tremolite in Fischer 344 rats. NTP Technical Report, National Toxicology Program, Research Triangle Park, NC, in press.
12. NTP. Carcinogenesis bioassay of amosite asbestos in Fischer 344 rats. NTP Technical Report, National Toxicology Program, Research Triangle Park, NC, in press.
13. Yu, B. P., Masoro, E. J., Murata, I., Bertrand, H. A., and Lynd, F. T. Life span study of SPF Fischer 344 rats fed *ad libitum* or restricted diets: longevity, growth, lean body mass and disease. J. Gerontol. 37: 130–141 (1982).
14. Haseman, J. K. Patterns of tumor incidence in two-year cancer bioassay feeding studies in Fischer 344 rats. Fund. Appl. Toxicol., in press.

15. Cooper, R. C., Murchio, J. C., and Paffenbarger, R. S. Asbestos in domestic water supplies for five California countries. Part II, EHS Publ. No. 79-1, School of Public Health, Univ. Calif. Berkeley, 247 (1979). In: EPA, Ambient Water Quality Criteria for Asbestos, EPA 440/5-80-022, U.S. EPA, Washington, DC, 1980.
16. Pozharisski, K. M. Morphology and morphogenesis of experimental epithelial tumors of the intestine. J. Natl. Cancer Inst. 54: 1115–1135 (1975).
17. Ward, J. M. Dose response to a single injection of azoxymethane in rats. Vet. Pathol. 12: 165–177 (1975).
18. Coleman, G. L., Barthold, S. W., Osbaldiston, G. W., Foster, S. J., and Jones, A. M. Pathological changes during aging in barrier-reared Fischer 344 male rats. J. Gerontol. 32: 258–278 (1977).
19. Goodman, D. G., Ward, J. M., Squire, R. A., Chu, K. C., and Linhart, M. S. Neoplastic and nonneoplastic lesions in aging F344 rats. Toxicol. Appl. Pharmacol. 48: 237–248 (1979).
20. Cook, P. M., and Olson, G. F. Ingested mineral fibers: elimination in human urine. Science 204: 195–198 (1979).
21. Sebastien, P., Masse, R., and Bignon, J. Recovery of ingested asbestos fibers from the gastrointestinal lymph in rats. Environ. Res. 22: 201–216 (1980).
22. Smith, W. E. Biological effects of tremolite talc on hamsters. U.S. Bur. Mines Inf. Circ. IC 8639: 43–48 (1974).
23. Donham, K. J., Berg, J. W., Will, L. A., and Leininger, J. R. The effects of long term ingestion of asbestos on the colon of F344 rats. Cancer 45: 1073–1084 (1980).
24. Gibel, W., Lohs, K. H., Horn, K. H., Wildner, G. P., and Hoffman, F. Investigation into a carcinogenic effect of asbestos filter material following oral intake in experimental animals. Arch. Geschwulstforsch. 46: 437–442 (1976).
25. Cunningham, H. M., Moodie, C. A., Lawrence, G. A., and Pontefract, R. D. Chronic effects of ingested asbestos in rats. Arch. Environ. Contam. Toxicol. 6: 507–513 (1977).
26. Gross, P., Harley, R. A., Swinberne, L. M., Davis, J. M. G., and Green, W. B. Ingested mineral fibres, do they penetrate tissue or cause cancer? Arch. Environ. Health 29: 341–347 (1974).
27. NTP. Carcinogenesis bioassay of amosite asbestos in Syrian golden hamsters. NTP Technical Report No. 249, National Toxicology Program, Research Triangle Park, NC, 1982.
28. NTP. Carcinogenesis bioassay of chrysotile asbestos in Syrian golden hamsters. NTP Technical Report No. 246, National Toxicology Program, Research Triangle Par' NC (1982).
29. Smith, W. E., Hubert, D. D., Sobel, H. J., Peters, E. T., and Doerfler, T. E. Health in experimental animals drinking water with and without amosite and other mineral particles. J. Environ. Pathol. Toxicol. 3: 277–300 (1980).
30. Hilding, A. C., Hilding, D. A., Larsen, D. M., and Aufderheide, A. C. Biological effects of ingested amosite asbestos, taconite tailings, diatomaceous earth and Lake Superior water in rats. Arch. Environ. Health 36: 298–303 (1981).
31. Ward, J. M., Frank, A. L., Wenk, M., Devor, D., and Tarone, R. E. Ingested asbestos and intestinal carcinogenesis in F344 rats. J. Environ. Pathol. Toxicol. 3: 301–312 (1980).

Environmental Health Perspectives
Vol. 53, pp. 45–48, 1983

Asbestos in Water Supplies of the United States

by James R. Millette,* Patrick J. Clark,* Judy Stober* and Montiel Rosenthal*

The review of available data on the concentrations of asbestos in U.S. water supplies suggests that the majority of water consumers are not exposed to asbestos concentrations over 1 million fibers/Liter. A few populations, however, may be exposed to concentrations over 1 billion fibers/L. Of the 538 water supplies for which waterborne asbestos data are available, 8% have concentrations of fibers over 10 million fibers/L.

The vast majority of asbestos fibers found in U.S. water supplies are under 5 μm in length.

Fiber Concentrations in Water

In 1980, two publications summarized the data on asbestos in drinking water that had been gathered from literature and other U.S. Environmental Protection Agency analytical records (*1,2*). The data are summarized in Table 1. Since that report, a number of studies have provided additional data on asbestos counts in drinking water.

McGuire and Bowers (*3*) have reported billions of chrysotile asbestos fibers in the California Aqueduct system. The reservoirs at the southern end of the State Water Project provide an opportunity for some of the asbestos particles to coagulate with other particles and settle to the bottom. However, concentrations as high as 1.3 billion fibers/L were reported at one water plant intake, and the maximum asbestos concentration in the effluent of one plant (i.e., in the drinking water) was 200 million fibers/L. The authors also reported variable asbestos concentrations up to 13 million fibers/L in the Colorado River water delivered to southern California. No evidence of amphibole fibers was found in either source of supply. Other data from the California Health Department indicate that some small water supplies in northern California have asbestos concentrations over 100 billion fibers/L (S. Hayward, Calif. Dept. of Health personal communication).

Table 1. Distribution of reported asbestos concentrations in drinking water from 406 cities in 47 states, Puerto Rico, and the District of Columbia.

Highest asbestos concentration, 10^6 fibers/L	Number of cities	Percentage
Below detectable limits	117	28.8
< 1	216	53.2
1–10	33	8.1
> 10	40	9.9
Total	406	100

The source is apparently natural erosion of serpentive minerals in the watershed. The water has very little turbidity, and no filtration treatment is employed for these northern California systems.

Data from both Canadian and U.S. sources (*4*) show concentrations of fibers as high as 100 billion fibers/L in the Sumas River, which flows into Canada from the State of Washington. The Yukon River, which is shared by Alaska and Canada, has been reported to have asbestos concentrations as high as 1 billion fibers/L for amphibole and 100 million fibers/L for chrysotile (*5*).

A report of asbestos in the groundwater of northern New Jersey was published by Germaine and Puffer (*6*) in 1981. They found 4.7 million fibers/L of crocidolite in the well water of the Mendham Borough water supply. Similar crocidolite fibers were found in samples of the local bedrock. In general, there are few reports of asbestos in groundwater sources. Earlier work showed groundwater asbestos concentrations as

*Toxicology and Microbiology Division, Health Effects Research Laboratory, U.S. EPA, Cincinnati, OH 45268.

high as 1 billion fibers/L in wells in New Mexico (*7*), but our laboratory could not confirm these results even though we used a more generally accepted technique of analysis.

EPA Region II has contracted to have 100 water supplies in New York and New Jersey analyzed for asbestos. The contractor's results are not yet available, but at least one supply has an asbestos concentration over 10 million fibers/L based on counts in Cincinnati for a portion of split samples used for quality control checking.

The cities of Chicago, Illinois, and Duluth, Minnesota, have continued to monitor their drinking water for asbestos as it comes from filtration plants. Neither reports any real variation from the values reported 4 yr ago. Both have asbestos concentrations under a million fibers per liter.

Cisterns receiving rainwater off asbestos-cement (AC) roofs have been found to contain levels of chrysotile asbestos as high as 500 million fibers/L (*8*). Asphalt shingles containing asbestos as a binder and used on roofs in the collection of rainwater were not found to contribute significant numbers of asbestos fibers to the cistern waters.

Discharges from asbestos processing and manufacturing plants can have very high asbestos concentrations as are being found in a new survey by the EPA Effluent Guidelines Division. There is no evidence, however, that any of these plants is directly contaminating drinking water supplies.

The study of the aggressive index (AI) as an indicator of a water's corrosivity toward AC pipe has continued. The AI is based on calcium carbonate saturation conditions and is derived from the sample pH, calcium hardness and total alkalinity according to the equation: $AI = pH + \log(AH)$, where A is the total alkalinity (expressed in mg/L $CaCO_3$) and H is the calcium hardness (expressed in mg/L $CaCO_3$). Values of AI below 10 are considered very aggressive, while values above 12 are considered nonaggressive. Analysis of water from AC pipe systems in Wisconsin where the water was aggressive showed fiber counts between 1 and 10 million fibers/L. Analyses of waters from towns in Virginia and Delaware with AC pipe and moderately aggressive waters did not show consistent fiber counts over 1 million fibers/L. In a paper published in 1981, Kanarek, Conforti, and Jackson (*9*) reported concentrations of asbestos as high as 34 million fibers/L in an AC distribution system with essentially nonaggressive water. The condition of the pipe surface was not investigated. In-house work by Schock and Buelow (*10*) has indicated some theoretical reasons why the AI will not always correctly predict fiber release from AC pipe. Other in-house research has suggested that iron, which is not considered in the AI, can, in concentrations at about 0.3 mg/L, coat the surface of a pipe in some way, even in the presence of an aggressive water. This coating does not necessarily prevent calcium from being depleted by the action of the aggressive water on the cement, but binds the fibers and prevents them from being eroded off the pipe. These data are consistent with an early Norwegian study (*11*) and some recent data from Germany (*12*) that showed protective iron-containing coats even in some aggressive water situations.

In response to a National Academy of Sciences recommendation that data were needed on the particulate concentration in typical U.S. drinking water, a survey was designed to obtain a representative picture of the particulates in U.S. water systems. Because of the time, cost and effort involved with the asbestos and other analyses, the survey was designed to sample only 100 U.S. water supplies. In order to get a representative sampling with only 100 collections, a stratified random sampling was done from the Community Water Systems Survey Database (*13*). This database list of 984 systems was originally obtained by a stratified random sampling of a list of over 40,000 utilities compiled by the USEPA Office of Drinking Water. A sample of 2139 utilities was randomly selected from the list, which was stratified geographically by EPA regions and demographically by seven population classifications of systems serving between 25 and 100,000 persons. Added to the sample was a list of all 232 utilities serving over 100,000 persons. Questionnaires were sent to these systems and the respondents, 948 systems, make up the Community Water Systems Survey Database.

From the database list stratified according to regions and three population groups, two groups of 100 systems were drawn as shown in Table 2. The second set of 100 acted as a reserve in the

Table 2. Distribution of systems by size and regions for 100 utility samples.[a]

U.S. EPA regions	Population 25–999	Population 1000–99,999	Population >100,000	Total
I and II	9 (88)	6 (60)	4 (41)	19 (189)
III and IV	10 (104)	6 (59)	7 (68)	23 (231)
V and VI	9 (87)	7 (70)	5 (44)	21 (201)
VII and VIII	10 (100)	6 (64)	3 (18)	19 (182)
IX and X	9 (96)	5 (53)	4 (32)	18 (181)
Total	49 (475)	30 (306)	23 (203)	100 (984)

[a]The number of water systems for each size and region in the Community Water Systems Database is given in parentheses.

event a utility in the first group could not be sampled. Table 2 shows that the distribution by cell of the 100 utility sample stratified for population size and geographic area, and the original database distribution by cell, represent about the same percentage of the total.

The water samples from residential drinking water taps were collected in the distribution systems of the utilities by EPA regional personnel, State health personnel, or local utility officials. The results of the asbestos analyses for these 100 utilities are shown in Table 3. When compared with the results from all asbestos analysis data available in 1982 (Table 4), the data from the representative sampling show a slightly lower percentage in the > 10 million fibers/L group. This may reflect the emphasis in the overall data on collecting more water samples from areas thought to have problems with asbestos. A survey of asbestos fibers in Canadian water supplies concluded that 5% of the public receive water with fiber concentrations exceeding 10×10^6 fibers/L (*14*). Consideration of the population served by the U.S. water supplies shown in Table 4 suggests that the percentage of U.S. population receiving water with fiber concentrations exceeding 10 million fibers/L is similar but somewhat less than the 5% figure determined for the Canadian population.

Sizes of Fibers

The length of a vast majority of asbestos fibers found in water supplies was less than 5 μm. Large numbers of waterborne chrysotile fibers have been sized in conjunction with an epidemiology study in California (*15*) and one in Puget Sound (Washington) (*16*). A comparison of the fiber size data in Table 5 shows that the San Francisco Bay Area water has a slightly higher percentage of longer fibers than that of the Puget Sound area.

Some caution is suggested when comparing these data, however, because the analytical methods used for sizing asbestos fibers in the Puget Sound area study (*17*) were more refined and perhaps more sensitive to small fibers than the methods used to characterize the asbestos fiber sizes of the Bay Area waters, which were studied at an earlier time (*18*).

Nonasbestos Fibers

For the survey of consumers' tapwater from 100 water utilities chosen to represent the size of population served and U.S. geographic area, data show a range in turbidity from 0.1 to 19 nephelometric turbidity units (NTU). The mean value for the set was 1.9 NTU. Particle counts from the same set of samples using a HIAC particle counter showed a range of 14,000 to 10,700,000 particles/L. The particles measured ranged in diameter from 2.5 to > 150 μm. The median particle size was 4 μm. Two nonasbestos mineral fibers, palygorskite and halloysite, have been found in concentrations over 1 million fibers/L in drinking water (*19*), but the occurrence of these forms of fibers is not widespread in water systems. Silicate algal scales, a biologically produced rigid fiber, can be found in a number of water systems (*20*). Glass fibers have not been identified in any water systems.

Conclusions

Additional data have not suggested that a change is necessary in the earlier conclusion (*1*) that the majority of U.S. water consumers are not exposed to constant concentrations of asbestos fibers over 1 million fibers/L. In some areas, however, people are exposed to concentrations of asbestos fibers over 1 billion fibers/L.

Table 3. 100-City survey: asbestos analysis.

Highest concentration, 10^6 fibers/L	Number of cities
Below detectable limits	61
< 1	27
1–10	7
> 10	5

Table 4. Asbestos in U.S. water, 538 cities.

Highest concentration, 10^6 fibers/L	Number of cities	%
Below detectable limits	187	34.8
< 1	264	49.1
1–10	41	7.6
> 10	46	8.6
Total	538	100.1

Table 5. Comparison of asbestos fiber lengths.

	California (Bay Area)	Washington (Puget Sound area)
N, fibers	7,375	6,977
Mean length, μm	1.35	0.6
Std. dev.	1.99	0.52
Median length, μm	1.0	0.5
Range, μm	0.1–59.0	0.1–9.5
Distribution		
90% <	2.2 μm	1.1 μm
75% <	1.5 μm	0.7 μm
25% <	0.6 μm	0.3 μm
Percentage ≥ 5 μm	2.3%	0.2%

The sizes of asbestos fibers in drinking waters vary depending on the source of the fibers. Generally, asbestos fibers found in drinking waters are less than 5 μm in length.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Millette, J. R., Clark, P. J., and Pansing, M. F. Exposure to asbestos from drinking water in the United States. Environmental Health Effects Research Report, Office of Research and Development, EPA-600/1-79-028, 1979.
2. Millette, J. R., Clark, P. J., Pansing, M. F., and Twyman, J. D. Concentration and size of asbestos in water supplies. Environ. Health Perspect. 34: 13–25 (1980).
3. McGuire, M. J., Bowers, A. E., and Bowers, D. A. Asbestos analysis case history: surface water supplies in Southern California. J. Am. Water Works Assoc. 74: 470–477 (1982).
4. Schreier, H., and Taylor, J. Asbestos Fibers in Receiving Waters. Technical Bulletin 117, Inland Waters Directorate, Environment Canada, Vancouver, British Columbia, Canada, 1980.
5. Marklund, D. R. Mineral Fiber Analysis of Yukon River Samples. Technical Report, U.S. Department of Interior, Fish and Wildlife Service, 1011 East Tudor Road, Anchorage, Alaska, 1981.
6. Germaine, M., and Puffer, J. H. Distribution of asbestos in the bedrock of the northern New Jersey area. Environ. Geol. 3: 337–351 (1981).
7. Oliver T., and Murr, L. E. An electron microscopic study of asbestiform fiber concentrations in Rio Grande Valley water supplies. J. Am. Water Works Assoc. 69: 428–431 (1976).
8. Millette, J. R., Boone, R., and Rosenthal, M. Asbestos in Cistern Water. Environ. Res. Brief, USEPA, Health Effects Research Laboratory, Cincinnati, 1980.
9. Kanarek, M. S., Conforti, P. M., and Jackson, L. A. Chrysotile asbestos fibers in drinking water from asbestos-cement pipe. Environ. Sci. Technol. 15: 923–925 (1981).
10. Schock, M. R., and Buelow, R. W. The behavior of asbestos-cement pipe under various water quality conditions: Part 2, theoretical considerations. J. Am. Water Works Assoc. 73: 636–651 (1981).
11. Kristiansen, H. Corrosion on asbestos-cement pipes. Vatten 83: 53–61 (1977).
12. Meyer, E. Untersuchungen zum Vorkommen von Asbestfasern in Trinkwasser in der Bundesrepublik Deutschland und gesundheitliche Bewertung der Ergebnisse (Studies of the presence of asbestos fibers in drinking water in the Federal Republice of Germany and a health evaluation of the results). GWF-Wasser/Abwasser 123: 85–96 (1982).
13. Herschell, C. Survey of Operating and Financial Characteristics of Commercial Water Systems. EPA Report #570-9-77-003, 1977.
14. Chatfield, E. J., and Dillon, M. J. A National Survey for Asbestos Fibers in Canadian Drinking Water Supplies. Department of National Health and Welfare, 79-EHD-34, Ottawa, Canada, 1979.
15. Kanarek, M. S., Conforti, P. M., Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer incidence in the San Francisco Bay Area. Am. J. Epidemiol. 112: 54–72 (1980).
16. Polissar, L., Severson, R. K., Boatman, E. S., and Thomas D. B. Cancer incidence in relation to asbestos in drinking water in the Puget Sound region. Am. J. Epidemiol. 116: 314–328 (1982).
17. Boatman, E. S. Analyzing asbestos fibers in water by means of transmission electron microscopy. J. Am. Water Works Assoc. 74: 533–536 (1982).
18. Cooper, R. C., and Murchio, J. C. Preliminary Studies of Asbestiform Fiber Concentrations in Domestic Water Supplies. Final Report to the Aerospace Medical Research Laboratory, Wright Patterson Air Force Base, Ohio. Available from NTIS, U.S. Department of Commerce #AD-A011-855, 1976.
19. Millette, J. R., Twyman, J. D., Hansen, E. C., Clark, P. J., and Pansing, M. F. Chrysotile, palygorskite, and halloysite in drinking water. Scan. Elec. Microsc. 1: 579–586 (1979).
20. Millette, J. R., Rosenthal, M. T., and Feldman. R. S. Asbestos analytical technique. In: Proceedings, American Water Works Association Water Quality Technical Conference, Miami, FL, December 1980.

Environmental Health Perspectives
Vol. 53, pp. 49–56, 1983

Critical Review of Epidemiologic Studies Related to Ingested Asbestos

by Gary M. Marsh*

Thirteen epidemiologic studies of ingested asbestos conducted in five areas of the United States and Canada were reviewed and evaluated for the definitiveness and applicability regarding the development of ambient water quality standards. One or more studies found male or female associations between asbestos in water supplies and cancer mortality (or incidence) due to neoplasms of the esophagus, stomach, small intestine, colon, rectum, gallbladder, pancreas, peritoneum, lungs, pleura, prostate, kidneys, brain, and thyroid, and also due to leukemia. Several methodologic weaknesses and limitations were found in each study, leading to the determination that no individual study or aggregation of studies exist that would establish risk levels from ingested asbestos. A binomial probability analysis of the eight independent studies suggested that, while the level of male-female agreement was generally low, the number of observed positive associations in males and females for neoplasms of the esophagus, stomach, pancreas, and prostate was unlikely to have been generated by chance factors alone, and thus, may have a biological basis related to ingested asbestos. Cancers of the small intestine and leukemia were implicated to a lesser degree in this analysis. The patterns of integrated findings for most gastrointestinal cancers were somewhat consistent with patterns observed among asbestos-exposed occupational groups, whereas the patterns found for pancreatic cancer, kidney cancer, and leukemia were not consistent. It was recommended that the integrated ecologic data to date be used to generate a rough priority of specific etiologic hypotheses that should be tested in the original settings or in independent study populations using studies designed at the more definitive individual level, such as case-control studies. The Bay Area (California) and Puget Sound (Washington) were deemed to be the existing study areas most suitable for future research.

Introduction

In 1982, the U.S. Environmental Protection Agency commissioned a critical review of the major epidemiologic studies that were germane to the question of possible adverse health effects caused by ingested asbestos. Thirteen published and unpublished studies conducted in five areas of the United States and Canada were included in the review (*1–13*). This paper presents the major findings and salient points of the more detailed review found elsewhere (*14*).

Background

The genesis of all of the studies included in this review was the 1973 discovery of large amounts of amphibole asbestos fibers in Lake Superior, the source of municipal water for Duluth, Minnesota, and five small communities on the lake shore. The first epidemiologic study to appear after this discovery was conducted in Duluth by Mason et al. In 1974 (*1*). Mason's study of 1950–1969 cancer mortality rates was followed by two studies of cancer incidence rates in Duluth, the first in 1976 by Levy et al. (*2*) and the second in 1981 by Sigurdson et al. (*3*). The two Connecticut studies of Harrington et al. in 1978 (*4*) and Meigs et al. in 1980 (*5*) were prompted by the possibility of studying reliable cancer incidence data over a 35-year period through the Connecticut Tumor Registry and linking these data with information collected on asbestos-cement pipe studies done by the U.S. EPA. In Canada, the mortality studies of Wigle in 1977 (*6*) and Toft et al. in 1981 (*7*) were induced by the extent of the asbestos mining done in Quebec and by environmental surveys that revealed high concentrations of asbestos fibers in the drinking water supplies of certain cities. The

*Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261.

San Francisco Bay Area cancer incidence studies of Kanarek et al. in 1980 (*8*), Conforti et al. in 1981 (*9*), and Tarter in 1981 (*10*) were motivated by the fact that several drinking water supplies come from aquifers or are stored in reservoirs that are exposed to serpentine, the parent rock form of chrysotile asbestos. The single unpublished epidemiologic study of cancer incidence conducted in Utah by Sadler et al. in 1981 (*11*) was based upon the fact that several Utah communities were known to have used predominantly asbestos-cement pipe for periods exceeding 20 years. Finally, the two studies of cancer incidence and mortality in the Puget Sound area by Severson et al. in 1979 (*12*) and Polissar et al. in 1982 (*13*) were motivated by the fact that three of the largest metropolitan areas of western Washington state have been almost constantly serviced since the early part of the 20th century by water supplies containing a wide range of chrysotile asbestos fibers.

Individual Reviews and Qualitative Integration of Findings

For each of the 13 studies, a determination was made of an overall positive, negative, or lack of association between ingested asbestos and cancer mortality or incidence. Determinations were based on general epidemiologic considerations while accounting for the strengths and weaknesses of the underlying study designs. Due to the subjectivity inherent in the assessment of research findings, the interpretations made were not always those of the authors cited.

Tables 1 and 2 show for gastrointestinal and nongastrointestinal cancer sites, respectively, a summary of results from the 13 studies. As shown here, one or more previous studies have found for males or females some association between asbestos in water supplies and cancer mortality (or incidence) for neoplasms of the esophagus (*1,8,9*), stomach (*1,2,6–9*), small intestine (*13*), colon

Table 1. Summary of studies of gastrointestinal cancer risk in relation to ingested asbestos by cancer site.[a]

Gastrointestinal cancer site, (ICD 7th revision codes)	Duluth			Connecticut		Quebec		Bay Area, CA			Utah	Puget Sound, WA	
	Mason (*1*)	Levy (*2*)	Sigurdson (*3*)	Harrington (*4*)	Meigs (*5*)	Wigle (*6*)	Toft (*7*)	Kanarek (*8*)	Conforti (*9*)	Tarter (*10*)	Sadler (*11*)	Severson (*12*)	Polissar (*13*)
All sites combined (150–159)	(+ +)	(− −)	(00)	ns	ns	(00)	(+0)	(+ +)	(+ +)	(+ +)	ns	(00)	ns
Esophagus (150)	(+ −)	(00)	(00)	ns	ns	(00)	(00)	(0+)	(+ +)	ns	ns	ns	(00)
Stomach (151)	(+ +)	(+0)	(00)	(00)	(00)	(+0)	(+0)	(+ +)	(+ +)	ns	(00)	(00)	(00)
Small intestine (152)	ns	(00)	(00)	ns	ns	ns	ns	(00)	(00)	ns	(00)	ns	(+ +)
Colon (153)	(00)	(− −)	(00)	(00)	(00)	(00)	(00)	(00)	(+0)	ns	(0−)	(− −)	(00)
Rectum (154)	(+ +)	(00)	(00)	(00)	(00)	(00)	(00)	(00)	(00)	ns	(00)	ns	(00)
Biliary passage/liver (155–156A)	(00)	(00)	(00)	ns	ns	ns	ns	(00)	(00)	ns	ns	ns	(00)
Gallbladder (155.1)	ns	(00)	(00)	ns	ns	ns	ns	(0+)	(00)	ns	(0+)	ns	(00)
Pancreas (157)	(0+)	(+ +)	(0+)	ns	(+0)	(0+)	(00)	(0+)	(+ +)	ns	(00)	ns	(00)
Peritoneum (158)	ns	(00)	(00)	ns	ns	ns	ns	(+ +)	(0+)	ns	(00)	ns	(00)

[a](Male, female) = association with ingested asbestos: + positive, 0 none, − negative, ns = not studied.

Table 2. Summary of studies of nongastrointestinal cancer risk in relation to ingested asbestos by cancer site.[a]

Nongastrointestinal cancer site (ICD 7th revision codes)	Duluth			Connecticut		Quebec		Bay Area, CA			Utah	Puget Sound, WA	
	Mason (*1*)	Levy (*2*)	Sigurdson (*3*)	Harrington (*4*)	Meigs (*5*)	Wigle (*6*)	Toft (*7*)	Kanarek (*8*)	Conforti (*9*)	Tarter (*10*)	Sadler (*11*)	Severson (*12*)	Polissar (*13*)
Buccal cavity and pharynx (140–148)	ns	ns	ns	ns	ns	(00)	(00)	ns	ns	ns	ns	ns	(00)
Bronchus, trachea, lungs (162, 163)	(+0)	ns	(00)	ns	(00)	(+0)	(+0)	(+0)	(00)	ns	ns	ns	(00)
Pleura (162.2)	ns	ns	ns	ns	ns	ns	ns	(0+)	(0+)	ns	ns	ns	ns
Prostate (177) (males only)	ns	ns	ns	ns	ns	0	0	0	+	ns	ns	ns	−
Kidneys (180)	ns	ns	ns	ns	(00)	(00)	(00)	(0+)	(00)	ns	(+0)	(00)	(00)
Bladder (181)	ns	ns	ns	ns	(00)	(00)	(00)	(00)	(00)	ns	ns	ns	(00)
Brain/CNS (193)	(00)	ns	ns	ns	ns	(00)	(00)	(00)	(00)	ns	ns	ns	(+ −)
Thyroid (194)	ns	ns	ns	ns	ns	ns	ns	(00)	(00)	ns	ns	ns	(+ +)
Leukemia, aleukemia (204)	(00)	ns	ns	ns	ns	(00)	(00)	(00)	(00)	ns	(+0)	ns	(+ −)

[a](Male, female) = association with ingested asbestos: + positive, 0 none, − negative, ns = not studied.

(*2,9,11, 12*), rectum (*1*), gallbladder (*8,11*), pancreas (*1–3,5,6,8,9*), peritoneum (*8,9*), bronchus, trachea, or lungs (*1,6–8*), pleura (*8, 9*), prostate (*9,13*), kidneys (*8,11*), brain or central nervous system (*13*), thyroid (*13*), and leukemia or aleukemia (*11,13*). The large variability in findings evident among the studies is matched by a considerable descrepancy in results for males and females within the 13 studies. Several factors might explain, at least in part, the internal and external inconsistencies in results.

First, the descrepant results may be due to differences in characteristics of asbestos exposure in the various study populations. These differences are summarized in Table 3. The relatively low number of positive associations found in Utah (*11*) and Connecticut (*4,5*) could be due to the low concentrations of asbestos in the drinking water or to the relatively short duration of community exposure in several study subareas. The virtual absence of positive findings in the most recent Duluth study (*3*) could also be due to relatively short duration of exposures as well as the amphibole fiber, which is fundamentally different from the chrysotile fibers found in the remaining study areas. By utilizing the differences in exposure characteristics, the three study areas associated with long duration of exposures (> 40 years) to chrysotile asbestos can be roughly ranked according to the concentration of fibers in their water systems. However, the resulting ranking, Bay Area (lowest), Puget Sound (intermediate), and Quebec (highest), does not appear to be related to the pattern of associations shown in Tables 1 and 2.

In addition to duration and intensity, it is also likely that other exposure factors, such as the characteristics of asbestos pipe used, the concentration of other possibly carcinogenic contaminants of water, and certain physical properties of asbestos fiber (e.g., length), vary among and within the six study areas.

As a second major factor, the different study designs employed in the various areas, coupled with the disparity in their underlying strengths and weaknesses, most likely also contributed to the observed variability in results. The most important methodologic weaknesses and limitations ascertained from the individual reviews are summarized in Table 4. The weaknesses are listed in approximate decreasing order of importance relative to their potential impact on the credibility and definitiveness of the findings.

By far the most serious limitation of all the studies conducted to date is that they are ecological or, more specifically, geographic correlation studies by design. This drawback alone does not permit a definitive conclusion to be made from any of the studies of the possible adverse health effects of ingested asbestos. The major drawback of ecological analysis for testing etiologic hypotheses is the potential for substantial bias in effect estimation. This problem, known as the "ecological fallacy," results from making a causal inference about individual phenomena on the bias of observations of groups. Theoretically, the bias resulting from ecological analysis can make an association appear stronger or weaker than it is at an individual level; however, in practice, this bias ordinarily exaggerates the magnitude of a true association, if one exists (*15–17*). Ecologic study bias can be minimized, for example, through the judicious application of ecologic regression techniques. Such techniques were employed, at least inpart, in the Connecticut study of Meigs (*5*), the three Bay Area studies (*8–10*), and the two Puget Sound studies (*12, 13*). However, the overall variability in results does not appear to be any less among or within these six studies compared to the remaining seven, which did not incorporate more refined ecologic analyses.

Much of the bias inherent in ecologic analysis results from the inability to control for confounding factors at the individual level. Table 4 shows that most of the studies reviewed did not directly control for confounding factors even at the group level. Notable exceptions are the Bay Area studies of Kanarek et al. (*8*) and Conforti et al. (*9*) and the two Puget Sound studies (*12,13*), which em-

Table 3. Characteristics of asbestos exposures in drinking water in various study populations.

Characteristic	Duluth	Connecticut	Quebec	Bay Area, CA	Utah	Puget Sound, WA
Type of asbestos	Amphibole	Chrysotile	Chrysotile	Chrysotile	Chrysotile	Chrysotile
Number of fibers/L[a,b]	1.0–30.0 – 10^6	BDL–0.7 – 10^6	1.1–1300 – 10^6	0.025–36 – 10^6	n.a.[b]	7.3–206.5 – 10^6
Population exposed	100,000	576,800	420,000	3,000,000	24,000	200,000
Maximum duration of exposure, yr	15–20	23–44	> 50	> 40	20–30	> 40

[a]BDL = below detectable limit.
[b]n.a. = data not available.

Table 4. Summary of methodologic weaknesses and limitations associated with various studies of ingested asbestos.[a]

	Duluth			Connecticut		Quebec		Bay Area, CA			Utah	Puget Sound, WA		Total
Weakness/limitation[b]	Mason (1)	Levy (2)	Sigurdson (3)	Harrington (4)	Meigs (5)	Wigle (6)	Toft (7)	Kanarek (8)	Conforti (9)	Tarter (10)	Sadler (11)	Severson (12)	Polissar (13)	across studies
Ecologic study design	*	*	*	*	*	*	*	*	*	*	*	*	*	13
Insufficient latency period	*	*	*	–	–	–	–	–	–	–	*	–	–	4
Death certificate data	*	–	–	–	–	*	*	–	–	–	–	–	–	3
Duration and/or intensity of exposure low	*	*	*	*	*	–	–	–	–	–	*	–	–	6
Uncontrolled confounding														
Race	–	*	*	*	*	*	*	–	–	*	*	*	*	10
Sex	–	–	–	–	–	–	–	–	–	*	–	–	–	1
Occupation	*	*	*	*	*	*	*	–	–	*	*	*	–	10
Socioeconomic status	*	*	*	*	–	*	*	–	–	*	–	–	–	7
Population density	*	*	*	–	–	*	*	*	*	*	–	*	*	10
Ethnicity	*	*	*	*	*	*	*	–	–	*	*	*	*	11
In/out migration	*	*	*	*	*	*	*	–	–	*	–	–	–	8
Personal habits	*	*	*	*	*	*	*	*	*	*	*	*	*	13
Absence (or incomplete) data on dose-response	*	*	*	*	*	–	–	–	–	–	*	*	*	8
Multiple comparisons problem	*	*	*	*	*	*	*	*	*	–	*	*	*	12
Insensitivity of summary statistics	*	*	*	*	*	–	–	*	*	*	–	*	*	10
Absence of historical asbestos exposure data	*	*	*	*	*	*	*	*	*	*	*	*	*	13
Use of at least one questionable statistical procedure	–	*	–	–	*	–	–	*	*	–	–	–	–	4
Total	14	15	14	12	12	11	11	7	7	11	10	10	9	

[a]Legend: asterisk (*) indicates presence of characteristic; minus (–) indicates absence of characteristic.
[b]In approximate decreasing order of relative impact on definitiveness of study results.

ployed relatively more sophisticated multivariate statistical analyses as an attempt to control for confounding at the group level. Only one study to date, that of Polissar et al. in 1982 (*13*), attempted to collect data on a confounding variable at the individual level; however, since this was done only for cancer cases and not controls, it was not possible to analyze the data on a more sensitive and reliable case-control basis.

Occupation was a particularly important confounding variable in the studies conducted in Quebec (*6,7*), the Bay Area (*8–10*), and Connecticut (*4,5*), since a substantial number of males are employed in the various asbestos-related industries within these areas. The confounding effects of occupation are particularly evident in the two Quebec studies (*6,7*), where positive associations for lung and stomach cancer were consistently confined to males.

Misclassification of asbestos exposures is another serious limitation of all the studies conducted to date. This misclassification results from several factors including: the basic ecologic design, which assigns specific exposures to an entire goegraphic area; tenuous assumptions regarding the extent of asbestos contamination from asbestos pipes; the lack of any reliable historical asbestos exposure data; and the in/out and daily mobility of the study populations.

It is also likely that many of the associations found among the 13 studies are simply chance occurrences arising from the large number of statistical comparisons that were generally made. Whenever a large number of significance tests are performed at a constant significance level, a certain number of tests will be significant by chance alone and the actual significance levels must be higher than those reported by the authors. Among the 13 studies reviewed, the number of separate statistical comparisons reported ranged from 33 to 336 with an average of 193. Therefore, at a 5% level of significance, the number of positive findings expected due to chance alone would range from approximately 2 to 17 with an average across the 13 studies of about 10. In other statistical terms, the probability that at least one of the n independent comparisons was due to chance alone ranged from 0.81 in a study reporting about 30 comparisons to virtual certainty in studies reporting 100 or more comparisons. (At the 5% level of significance, the probability of falsely claiming statistical significance in at least one of n independent comparisons is $1\text{-}0.95^n$.

Objective Integration of Findings

In order to objectively evaluate the extent to which the pattern of findings to date may be due

to chance factors, and to better assess the degree of interstudy consistency, a probability analysis was performed for each cancer site, which was examined in at least four independent studies. The studies of Levy et al. in 1976 (*2*), Harrington et al. in 1978 (*4*), Kanerek et al. in 1980 (*8*) and Severson in 1979 (*12*) were not considered independent studies, since they provided no unique information in light of the subsequently updated and improved analyses of Sigurdson et al. in 1981 (*3*), Meigs et al. in 1981 (*5*), Conforti et al. in 1981 (*9*) and Polissar et al. in 1982 (*13*), respectively. In addition, the study of Tarter in 1981 (*10*) was not included in the probability analysis, since no cancer site-specific results were shown.

For each cancer site, the probability analysis consisted of first casting the independent study results of Tables 1 and 2 into a 2 × 2 contingency table of male-female results as shown in Table 5.

The next step in the analysis was to calculate for each cancer site the probability of jointly observing in $n_{..}$ independent studies, $n_{1.}$ or more positive associations in males and $n_{.1}$ or more positive associations in females. This was done assuming that for males and females the probability of observing a positive association in a given independent study due to chance alone is $p = 0.05$, and the probability of observing no association is $(1 - p) = 0.95$. Designating M and F to represent the events of observing a positive association in males and females, respectively, and assuming that outcomes in males and females are independent events, the probability of the joint event (known as a large deviation probability, P_D) can be calculated as the product of two individual cumulative binomial probabilities as follows:

$$P_D = P(M \geq n_{1.}) \cdot P(F \geq n_{.1})$$

$$= \left\{ \sum_{i = n_{1.}}^{n_{..}} \binom{n_{..}}{i} p^i (1 - p)^{n_{..} - i} \right\} \left\{ \sum_{j = n_{.1}}^{n_{..}} \binom{n_{..}}{j} p^j (1 - p)^{n_{..} - j} \right\}$$

P_D was also calculated by using the binomial parameter $p = 0.10$ assuming that a predetermined significance level of $p = 0.05$ would have actually been higher for any individual observed positive association due to the very large number of statistical comparisons that were made in most of the independent studies. Very small values of P_D (less that 0.05, for example) for a given cancer site suggest that the number of observed positive associations in males and females across several independent studies was unlikely to have been generated by chance factors alone, and, therefore, may have a biological basis related to ingested asbestos. The P_D value as calculated above does not, however, take into account the degree of association between male and female findings. Unfortunately, the very small numbers of independent studies showing results for specific cancer sites precluded the calculation of any reliable measure of association. However, in order to provide at least a crude objective comparison of the level of agreement between male and female findings, the well-known phi coefficient given as

$$\phi = (\chi_u^2 / n_{..})^{1/2}$$

was computed where χ_u^2 is the uncorrected chi-square statistic tabulated from the above 2 × 2 contingency table as

$$\chi_u^2 = \frac{n_{..} (n_{11} n_{22} - n_{12} n_{21})^2}{n_{1.} n_{2.} n_{.1} n_{.2}}$$

Values of close to zero indicate little, if any, association, whereas values close to unity indicate almost perfect predictability. By definition, the phi coefficient cannot be determined whenever $n_{1.}$, $n_{.1}$, $n_{2.}$, or $n_{.2}$ is equal to zero. Finally, the strength of the association between male and female findings was assessed through the use of the Fisher-Irwin exact test (*18*).

Table 6 shows the results of the probability analysis for gastrointestinal and nongastrointestinal cancer sites, which were examined in at least four independent studies. Only five of the 14 sites shown in Table 6 (esophagus, stomach, pancreas, lungs, and prostate) are associated with P_D values that range consistently below or near a probability level as low as 0.05, for example. However, as shown by the ϕ value and corresponding Fisher-Irwin probability, or by inspection of the outcome frequencies, the level of agreement between male and female findings for these cancers is generally moderate to low. Specifically, positive associations were jointly observed in males and females in only one of six studies of esophageal cancer, two of eight studies of stomach cancer, and one of eight studies of pancreatic cancer. It was not possible to quantify the level of male-female agreement for lung or several other cancers due to the presence of one or more zero marginal totals.

Two additional neoplasms (small intestine, and leukemia/aleukemia) are associated with P_D val-

Table 5.

		Female association		
		(+)	(0 or −)	Total
Male association	(+)	n_{11}	n_{12}	$n_{1.}$
	(0 or −)	n_{21}	n_{22}	$n_{2.}$
	Total	$n_{.1}$	$n_{.2}$	$n_{..}$

Table 6. Summary of male-female associations in independent studies by cancer site.

Cancer site	No. of independent studies ($n_{..}$)	Outcome (++) (n_{11})	(+0) (n_{12})	(0+) (n_{21})	(00) (n_{22})	Total male (+) ($n_{1.}$)	Total female (+) ($n_{.1}$)	Large deviation probability (P_D) (p = 0.05)	(p = 0.10)	Index of association ϕ	Fisher-Irwin probability
Gastrointestinal											
Esophagus	6	1	1	0	4	2	1	0.0087	0.0535	0.63	0.33
Stomach	8	2	2	0	4	4	2	<0.0001	0.0009	0.55	0.21
Small intestine	4	1	0	0	3	1	1	0.0344	0.1183	1.00	0.25
Colon	8	0	1	0	7	1	1	0.3366	0.5695	NC[a]	NC
Rectum	8	1	0	0	7	1	1	0.1132	0.3243	1.00	0.12
Biliary passages/liver	4	0	0	0	4	0	0	1.00	1.00	NC	NC
Gallbladder	4	0	0	1	3	0	1	0.1855	0.3439	NC	NC
Pancreas	8	1	1	3	3	2	4	<0.0001	0.0009	0.0	0.78
Peritoneum	4	0	0	1	3	0	1	0.1855	0.3439	NC	NC
Nongastrointestinal											
Bronchus, trachea, lungs	7	0	3	0	4	3	0	0.0038	0.0257	NC	NC
Kidneys	6	0	1	0	5	1	0	0.2649	0.4686	NC	NC
Bladder	5	0	0	0	5	0	0	1.00	1.00	NC	NC
Brain/CNS	5	0	1	0	4	1	0	0.2262	0.4095	NC	NC
Leukemia/aleukemia	6	0	2	0	4	2	0	0.0328	0.1143	NC	NC
Prostate (males only)	4	–	–	–	–	2	–	0.0140	0.0523	–	–

[a]NC = not calculated due to presence of one or more zero marginal frequencies.

ues below 0.05 when based on the binomial parameter p = 2.05, but exceed P_D = 0.05 when based on the more conservative p = 0.10. While still based on very small numbers of independent studies, the P_D values for the remaining cancer sites examined suggest that the number of positive male and female associations, if any, observed for these cancers is more likely to represent chance phenomena.

It should be recognized that, next to very small sample size, the most severe limitation of the above probability analysis was the necessity to assume that the $n_{..}$ independent studies provided qualitatively and quantitatively equivalent information toward the integration of findings for any given cancer site. Therefore, the results of the probability analysis should not be regarded as conclusive, but rather should serve as a rough guide for the future direction and emphasis of research.

Relationship to Occupational Studies

The pattern of integrated findings presented for gastrointestinal cancers is somewhat consistent with patterns observed among workers occupationally exposed to asbestos. Epidemiologic studies of several occupational groups exposed to asbestos have shown an increased incidence of cancer of the esophagus, stomach, colon, and rectum and of peritoneal mesotheliomas (*19–22*). Furthermore, as noted by Mason et al. in 1974 (*1*), certain studies of asbestos installation workers in the United States have shown cancer of the upper gastrointestinal tract to be in far greater excess than cancer of the colon and rectum. This same feature is suggested in Table 6, where upper gastrointestinal cancers are among the strongest positive results, whereas positive associations for colon and rectal cancer are virtually nonexistent. The relatively large number of independent positive associations found for pancreatic cancer suggests a possible link with ingested asbestos, although most occupational studies have not implicated this cancer site.

With respect to nongastrointestinal neoplasms, an increased risk for cancer of the kidneys has been found in a recent occupational study of insulation workers (*23*). A biological basis for this risk has been described by Cook and Olson (*24*). However, as shown in Table 6, kidney cancer was observed in excess among males in only one of the six independent studies reviewed that examined this anatomic site. It is uncertain whether the marginally significant number of leukemia/aleukemia and prostatic cancer findings are related to ingested asbestos, since these are generally not considered in occupational studies as sites where asbestos-induced cancers would occur.

Recommendations for Future Research

Although no individual study or aggregation of studies exists that would establish risk levels from the ingestion of asbestos, the studies to date do provide extremely valuable information that should be carefully considered when developing the protocols of future research.

First, the integrated study findings can be used to generate a rough priority of specific etiologic

hypotheses that could be tested in the original settings or in independent study populations via more sensitive and reliable epidemiologic designs. The foremost intensive efforts should be made to further study the relationahip of ingested asbestos to the gastrointestinal neoplasms that displayed the most suggestive findings in the ecologic studies. In approximate order of importance, these would be stomach, pancreas, esophagus, and small intestine. The outcomes of these endeavors could be used to determine whether additional studies of other gastrointestinal neoplasms were warranted. In addition, the integrated findings for prostatic cancer, although less biologically plausible, were sufficiently disconcerting to make the relationship of ingested asbestos to this male neoplasm the subject of another more intensive study.

Second, the existing studies have produced a virtual checklist of methodologic limitations and uncertainties that should be avoided or controlled to the fullest extent possible in all future research efforts. Many of the aforementioned weaknesses and limitations can be avoided by simply choosing more suitable geographic areas for study. The "ideal" study area would be one associated with a long history of a wide range of asbestos exposures of known and well-documented magnitude. This would allow a sufficient latency period for the development of disease and would permit a more sensitive and accurate assessment to be made of dose-response relationships. While none of the areas studied to date can be necessarily considered as ideal, the Bay Area and Puget Sound are relatively the most suitable areas for future research. Further studies in new independent areas should also be considered since this will improve the ability to evaluate the strength and consistency of findings statistically.

Many of the other methodologic limitations are features of the underlying ecologic study designs that were employed. The ability to make a causal inference from ecologic data often can be enhanced using more sophisticated analytical techniques. There will always remain an element of uncertainty, however, until the etiologic hypotheses generated from ecologic studies are tested more definitively at the individual rather than group level.

The diseases implicated in the ecologic studies to date are relatively rare in the general population and are associated with long incubation periods. Thus, the retrospective approach is apropriate using, for example, either an unmatched or matched individual case-control design. Basically, a case-control study would compare the ingested asbestos exposures of individual site-specific cases of cancer (incidence or mortality) with unmatched or matched controls. This approach would enable a much more precise measurement of confounding factors such as occupation, socioeconomic status, tobacco and alcohol consumption, dietary habits, and migration history through personal interviews with each case (or next of kin) and control. While the level of asbestos exposure would probably still be determined by geographic residence, the duration of exposure could be much more accurately measured and controlled by determining length of residence. In addition, individual differences in water ingestion habits due to daily mobility and other personal factors could be assessed during the interviews. It is very important that the case-control protocol include procedures for checking the reliability and validity of the methods used to ascertain historical ingested asbestos exposures.

The number of subjects to be selected for a study of a specific disease-exposure relationship will be a fundamental consideration in planning future studies. Basically, an answer to the question of how many subjects should be selected for a case-control study, for example, depends on the specification of four values: the relative frequency of exposure among controls in the target population p_0; a hypothesized relative risk associated with exposure that would have sufficient biologic or public health importance to warrant its detection R; the desired level of significance α; and the desired study power, $(1 - \beta)$ (*25*). As an illustrative example, Table 7 shows for a standard unmatched case-control design the required sample size n (per group) under the conventional $\alpha = 0.05$ (one-sided), $\beta = 0.20$, and for selected values of R and p_0. In the study areas recommended for individual case-control analysis (the Bay Area and Puget Sound), relative risk levels R for gastrointestinal cancer were generally found by ecologic analysis to be only moderately elevated ($R \approx 1.1$–2.0), if elevated at all. This is likely to be the case in most areas unless levels of asbestos in the drinking water are inordinately high. In order to detect these putative moderate elevations in relative risk at acceptable statistical error levels (α, β), it will be necessary, as shown in Table 7, to study literally hundreds of cases and controls. This may be a serious drawback when studying the rarer forms of gastrointestinal cancer (e.g., small intestine), for it may be difficult to observe and locate the required number of cases during a reasonable period of time. For some cancers, therefore, it may be necessary to accept somewhat higher levels of statistical errors in order to

Table 7. Unmatched case-control sample sizes needed in each group for $\alpha = 0.05$ (one-sided) and $\beta = 0.20$.

Relative risk R	Proportion of controls exposed, p_0			
	0.25	0.50	0.75	0.90
1.2	1890	1484	2071	4431
1.5	365	303	447	987
1.7	208	179	272	610
2.0	119	107	168	385
2.5	66	63	104	246
3.0	45	45	78	187
5.0	20	24	45	114

test the null hypothesis of no risk with the available number of cases.

In conclusion, there is no question that studies designed at the individual level, such as case-control studies, are now needed to establish firmly risk levels to ingested asbestos. However, as illustrated above, the costs of reliably establishing these risk levels will be high, a fact that should be recognized by the sponsors and investigators of future research in this area.

Funding for this work was provided by the Center for Environmental Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, and the U.S. Environmental Protection Agency, Cincinnati, Ohio, under Cooperative Agreement No. 806815.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Mason, T. J., McKay, F. W., and Miller, R. W. Asbestos-like fibers in Duluth water supply. J. Am. Med. Assoc. 228: 1019–1020 (1974).
2. Levy, B. S., Sigurdson, J., Mandel, E., Landon, E., and Pearson, J. Investigating possible effects of asbestos in city water: Surveillance of gastrointestinal cancer incidence in Duluth, MN. Am. J. Epidemiol. 103: 362–368 (1976).
3. Sigurdson, E. E., Levy, B. S., Mandel, J., McHugh, R., Michienzi, L. J., Jagger, H., and Pearson, J. Cancer morbidity investigations: Lessons from the Duluth study of possible effects of asbestos in drinking water. Environ. Res. 25: 50–61 (1981).
4. Harrington, J. M., Craun, G., Meigs, J. W., Landrigan, P. J., Flannery, J. T., and Woodhull, R. S. An investigation of the use of asbestos cement pipe for public water supply and the incidence of gastrointestinal cancer in Connecticut, 1935–1973. Am. J. Epidemiol. 107: 96–103 (1978).
5. Meigs, J. W., Walter, S., Heston, J., Millette, J. R., Craun, G. F., Woodhull, R. S., and Flannery, J. T. Asbestos-cement pipe and cancer in Connecticut, 1955–1974. J. Environ Res. 42: 187–191 (1980).
6. Wigle, D. T. Cancer mortality in relation to asbestos in municipal water supplies. Arch. Environ. Health 32: 185–189 (1977).
7. Toft, P., Wigle, D. T., Meranger, J. C. and Mao, Y. Asbestos and drinking water in Canada. Sci. Total Environ. 18: 77–89 (1981).
8. Kanarek, M. S., Conforti, P., Jackson, L., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer incidence in the San Francisco Bay Area. Am. J. Epidemiol. 112: 54–72 (1980).
9. Conforti, P. M., Kanarek, M., Jackson, L., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer in the San Francisco Bay Area: 1969–1974 incidence. J. Chronic Dis. 34: 211–224 (1981).
10. Tarter, M. E. Pattern recognition in the context of an asbestos cancer threshold study. Preprint, Proceedings of the 13th Annual Symposium on the Interface: Computer Sci. Statistic. (1981).
11. Sadler, T. D., Rom, W. N., Lyon, J. L., and Mason, J. O. The use of asbestos-cement pipe for public water supply and the incidence of cancer in selected communities in Utah, 1967–1976. Graduate thesis, University of Utah, 1981.
12. Severson, R. A study of the effects of asbestos in drinking water and cancer incidence in the Puget Sound region. M.S. Thesis, Univ. of Wash., Seattle, Washington (1979).
13. Polissar, L., Severson, R. K., Boatman, E. S., and Thomas, D. B. Cancer incidence in relation to asbestos in drinking water in the Puget Sound Region. Am. J. Epidemiol. 116: 314–328 (1982).
14. Marsh, G. M. Review of epidemiologic studies related to ingested asbestos. Report submitted to the Center for Environmental Epidemiology, Graduate School of Public Health, University of Pittsburgh and the U.S. Environmental Protection Agency, October 12, 1982.
15. Langbein, L. I., and Lichtman, A. J. Ecological Inference. Sage Publications, Beverly Hills, CA, 1978.
16. Duncan, O. D., Cuzzort, R. P., and Duncan, B. Statistical Geography: Problems in Analyzing Areal Data. Greenwood Press, Westpoint, CT, 1961.
17. Valkonen, T. Individual and structural effects in ecological research. In: Social Ecology (M. Dogan and S. Rokbran, Eds.), MIT Press, Cambridge, MA, 1969.
18. Hollander, M., and Wolfe, D. A. Nonparametric Statistical Methods. John Wiley and Sons, New York, 1973.
19. Selikoff I. J., Churg, J., and Hammond, E. C. Asbestos exposure and neoplasia. J. Am. Med. Assoc. 188: 22–26 (1964).
20. Enterline, P. E., and Kendrick, M. A. Asbestos dust exposures at various levels and mortality. Arch. Environ. Health 15: 181–186 (1967).
21. Newhouse, M. L. A study of the mortality of workers in an asbestos factory. Brit. J. Ind. Med. 26: 294–301 (1969).
22. Elmes, P. C., and Simpson M. J. C. Insulation workers in Belfast. A further study of mortality due to asbestos exposure. Brit. J. Ind. Med. 34: 174–180 (1977).
23. Selikoff, I. J., Hammond, E. L., and Seidman, H. Mortality experience of insulation workers in the United States and Canada, 1943–1977. Ann. N.Y. Acad. Sci. 330: 91–116 (1979).
24. Cook, P. M., and Olson, G. F. Ingested mineral fibers: elimination in human urine. Science 204: 195–198 (1979).
25. Schlesslman, J. J. Sample size requirements in cohort and case-control studies of disease. Am. J. Epidemiol. 99: 381–384 (1974).

Environmental Health Perspectives
Vol. 53, pp. 57–60, 1983

Cancer Risk from Asbestos in Drinking Water: Summary of a Case-Control Study in Western Washington

by Lincoln Polissar,*† Richard K. Severson*† and Edwin S. Boatman†

We conducted a case-control, interview-based study of the risk of developing cancer from asbestos in drinking water. An area that included Everett, Washington, was selected for the study because of the unusually high concentration of chrysotile asbestos in drinking water from the Sultan River.

Through a population-based tumor registry, 382 individuals with cancer of the buccal cavity, pharynx, respiratory system, digestive system, bladder, or kidneys, diagnosed between 1977 and 1980, were identified, and they or their next of kin were interviewed. We conducted validation checks of our interviews, including a comparison with secondary sources.

Data on asbestos exposure were collected based on residence and workplace history, and on individual water consumption. Logistic regression was used to estimate cancer risk. We found no convincing evidence for increased cancer risk from imbibed asbestos. Confidence intervals for relative risks for almost all sites included unity. There were significantly elevated risks only for male stomach and male pharyngeal cancer, but these sex-inconsistent results, based on small numbers of cases, are probably due to other factors.

Our interest in conducting a study of cancer incidence and waterborne asbestos was stimulated, as was that of previous researchers, by the known carcinogenic effect of inhaled asbestos (*1,2*) and the discovery of asbestos fibers in a number of public water supplies (*3–5*).

All previous studies of the potential carcinogenic effect of imbibed asbestos, however, have been ecologic ones (*6–13*). In almost all of the previous studies, asbestos exposure was imputed from residence at the particular moment of the study, with no measurement of the duration or degree of individual exposure, or the pattern of individual migration.

In contrast, our study, which is described more fully elsewhere (*14*), determined individual exposure through in-person interviews with cancer cases or their next of kin and with members of a control group.

Materials and Methods

Study Area

We chose cases and controls from the Everett, WA, area, which has used the Sultan River as a source of drinking water since 1918. Sultan River tapwater has concentrations of chrysotile asbestos around 200 million fibers/L, among the highest in the United States (*5,15*).

Since a large volume of migration into the study area could produce a population with small cumulative exposures, we limited our study area to 1970 census tracts with lower than average migration rates.

Cases

Data on incident cancer cases were obtained through the Cancer Surveillance System (CSS), a population-based tumor registry that covers 13

*Fred Hutchinson Cancer Research Center, 1124 Columbia St., Seattle, WA 98104.
†School of Public Health and Community Medicine, University of Washington, Seattle, WA 98195.

counties and a population of nearly 3 million in western Washington. The CSS is part of the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute (*16*).

All invasive or *in situ* cases of cancer of the buccal cavity (excluding the lip), pharynx, respiratory system, digestive system, bladder, or kidney newly diagnosed between November 1977 and December 1980 were identified. The sites were chosen primarily on the basis of preceding studies, particularly the reported positive findings of Kanarek et al. (*12*), sites previously implicated in studies of airborne asbestos, sites that might be involved in elimination of asbestos fibers, and sites along the alimentary tract.

Only those individuals between 40 and 79 yr of age who resided in the eligible census tracts at the time of diagnosis were included in this study; both living and deceased were included.

Controls

We chose unmatched population controls of the same age range (40–79) and from the same group of 25 census tracts as the cases, using standard area sampling methods. We attempted to schedule an interview with each person of eligible age in 649 households selected.

Questionnaire

In-person interviews with subjects or next of kin focused on residence and workplace history, and the amount of water consumed by each individual. Information on other risk factors was also collected.

Quality Control Procedures

Quality control procedures included interview validation, independent recoding, and comparison of interview information with secondary sources. Since extensive concern has been expressed over the use of surrogate interviews for deceased cases (*17*), we compared a sample of subjects' residence and occupation histories with listings in independent, annually published Everett City Directories (*18*) from 1930 to 1980.

Exposure to Imbibed Asbestos

By meeting with representatives of water companies, we learned the history of water district boundaries and could determine the source of tapwater (and the asbestos concentration) for any given location and date.

We then calculated four separate variables expressing cumulative exposure to imbibed asbestos for each subject. The calculations were based on each subject's residence and workplace history and on typical water consumption from all sources "five years ago." The first variable was based only on residence history and workplace history. The second variable was obtained by multiplying the first variable by the total amount of water intake as determined from the water consumption questions.

The second pair of variables was calculated in a similar fashion, except that we ignored all residence and work locations during a presumed 10-yr latent period prior to diagnosis or interview.

Statistical Methods

We estimated cancer risk by fitting a logistic regression model (*19*). The dependent variable was a dichotomous indicator of a specific cancer site versus control status. Predictor variables always included age and cumulative exposure to asbestos in water. Other covariates included well-established risk factors such as smoking. For each site, each sex was analyzed separately except for a few sites in which small numbers required a joint analysis. In such cases, sex was included as a covariate. Cross tabulation and other methods were used to check the logistic regression results.

We also estimated the statistical power of the logistic regression method to detect cancer risk.

Results

Interviewing Completion Rates

Of the total 445 eligible cases, the overall refusal rate was 13.5%. Of the total 549 eligible controls, the refusal rate was 11.7%.

After the interview and before analysis, we limited the study to white subjects, since we found only one nonwhite subject. Two additional interviews with the next of kin of subjects were also excluded because virtually all answers were "unknown." The final roster for analysis was 382 cases and 462 controls.

Quality Control Checks

Validation checks by supervisors showed that the data collected by the interviewers were highly reliable. A comparison of coding and independent recoding for a sample of subjects turned up an average of only one disagreement per lengthy questionnaire. Comparison of residence and employer histories with information from annual city directories also showed low disagreement rates that were similar across interviews with

living cases, next of kin of deceased cases, and controls.

Exposure Levels

The amount of exposure to chrysotile asbestos in drinking water was extremely similar between cases and controls. For example, 23% of the cases and 21% of the controls had 30 yr or more of exposure to Sultan River drinking water. If a 10-yr latent period is taken into account, exposure is still similar. The amount of water intake per week was also very similar between cases and controls.

Relative Risk

The validity of the data collected and the logistic regression analysis are supported by reconfirmation of other known risk factors. The logistic regression model, for example, produces coefficients that are generally positive for age (indicating increasing cancer risk with age) and smoking, and are in the right direction for other risk factors.

Summarizing our findings for imbibed asbestos, we found very few elevated risks of statistical significance. Considering the relative risk for each of the sites and for each of the four asbestos exposure variables, we found no instance in which the risk was elevated for both males and females. The only statistically significant ($p < 0.05$) elevated risks occurred for male pharynx and male stomach. The observed number of "significant" results is not surprising considering the number of comparisons made. As a summary measure, we calculated the relative risk from the logistic regression model for a 20-yr cumulative use of the Sultan River tapwater versus no exposure. On this basis, the male pharynx relative risk (RR) was 2.99 (lower 95% confidence bound = 1.43) with RR = 0.26 for females. The male stomach relative risk was 1.71 (lower 95% confidence bound = 1.06) with RR = 0.65 for females. These estimated risks were similar for each of the four asbestos exposure variables. For males, the stomach cancer risks are bases on eight cases, and the pharyngeal cancer risks are based on four cases.

The pancreas, a site that we previously noted was most consistently implicated by the various ecologic studies (*13*), appears in our study with a sex-inconsistent negative risk for males and a positive risk for females. None of the risks was statistically significant at the 5% level.

Sometimes risks become evident only at very high levels of exposure. To test this, we calculated relative risks for persons with 30 yr or more of Sultan River exposure and compared them with the risks for persons with 5 yr or fewer of exposure. This analysis included only the digestive system of males and females and all study sites grouped for both sexes. The calculated risks were nonsignificant and were similar to those based on all exposure.

Power Considerations

For single-sex analyses, the minimum risk that could be detected at the 5% significance level with 80% probability was under 2.0 for each sex for the following sites or site groups: all study sites combined, digestive system, respiratory system, colon and lung.

Discussion

Some limitations of the study are worth noting. We lack numbers of cases to make really solid judgment about risk for the rarer sites, such as the kidneys and gallbladder. It does appear, however, that the risk is unlikely to be large for any of the sites we have studied, due to low calculated risks or to sex-inconsistent results.

Another limitation is the effect of a possible long latent period. The latent period for inhaled asbestos appears to be about 30 yr (*20*). Exposure prior to the latent period would increase the power of the study. We note that about 25% of the cases and controls had an onset of exposure beginning at least 40 yr ago, even though we chose the more stable census tracts in our area. The migration habits of U.S. residents generally result in smaller exposures to geographically fixed sources compared to such exposures in more stable populations.

Finally, the use of next of kin for deceased cases, plus a few proxies for the eligible controls who were unavailable for interview, may have introduced some bias unknown to us, despite our verification checks.

Balancing these limitations of the study, however, are a number of strengths. The exposures are individually based and appear to be accurately measured. The case and control refusal rates were low, and the cases, from a population-based tumor registry, are representative of all cases in a community that does not have atypical features. The controls, which had been carefully drawn from the same population that contributed the cases, were interviewed during the same period. The community chosen for study has a very high level of asbestos in water and has apparently had this level for at least 60 yr.

We would suggest that the next step in this line of research should be to discuss the feasibility and advisability of attempting to detect very low risks or to detect risks for rare sites. We propose that additional studies not be undertaken hastily, since the ability to detect the risks involved may not be available in current epidemiologic methods except at great effort or expense.

We would like to thank staff of the water departments in King, Pierce and Snohomish Counties for their generous cooperation in all aspects of the study. James Millette and Roy Jones of the Environmental Protection Agency were of invaluable assistance. Our thanks to Kathy Prindle for technical assistance, to our Field Studies staff for excellent support, and to Claire Frost for editorial help. Funding for this study was provided by the Environmental Protection Agency under Grant No. 805846.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Selikoff, L. J., Churg, J., and Hammond, E. C. Asbestos exposure and neoplasia. J. Am. Med. Assoc. 188: 22–26(1964).
2. Enterline, P. E., and Kendrick, M. A. Asbestos-dust exposure at various levels and mortality. Arch. Environ. Health 15: 181–186(1967).
3. Cunningham, H. M., and Pontefract, R. Asbestos fibres in beverages and drinking water. Nature 232: 332–333(1971).
4. Cook, P. M., Glass, G. E., and Tucker, J. H. Asbestiform amphibole minerals: Detection and measurement of high concentrations in municipal water supplies. Science 185: 853–855(1974).
5. Millette, J. R., Clark, P. J., Pansing, M. F., and Twyman, J. D. Concentration and size of asbestos in water supplies. Environ. Health Perspect. 34: 13–25(1980).
6. Mason, T. J., McKay, R. W., and Miller, R. W. Asbestos-like fibers in Duluth water supply; relation to cancer mortality. J. Am. Med. Assoc. 228: 1019–1020(1974).
7. Levy, B. S., Sigurdson, E., Mandel, J., Laudon, E., and Pearson, J. Investigation of possible effects of asbestos in city water: Surveillance of gastro-intestinal cancer incidence in Duluth Minnesota. Am. J. Epidemiol. 103: 362–368(1976).
8. Wigle, D. T. Cancer mortality in relation to asbestos in municipal water supplies. Arch. Environ. Health 32: 185–190(1977).
9. Harrington, M. J., Craun, G. F., Meigs, J. W., Landrigan, P. J., Flannery, J. T., and Woodhull, R. S. An investigation of the use of asbestos cement pipe for public water supply and the incidence of gastrointestinal cancer in Connecticut, 1935-1973. Am. J. Epidemiol. 107: 96–103(1978).
10. Meigs, J. W., Walter, S. D., Heston, J. F., Millette, J. R., Craun, G. F., Woodhull, R. S., and Flannery, J. T. Asbestos cement pipe and cancer in Connecticut 1955–1974. J. Environ. Health 42: 187–191(1980).
11. Conforti, P. M., Kanarek, M. S., Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer incidence in the San Francisco Bay Area: 1969–1974. J. Chronic. Dis. 34: 211–224(1981).
12. Kanarek, M. S., Conforti, P. M. Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer incidence in the San Francisco Bay Area. Am. J. Epidemiol. 112: 54–72(1980).
13. Polissar, L., Severson, R. K., Boatman, E. S., and Thomas, D. B. Cancer incidence in relation to asbestos in drinking water in the Puget Sound region. Am. J. Epidemiol. 116: 314–328(1982).
14. Polissar, L., Severson, R. K., and Boatman, E. S. A case control study of asbestos in drinking water and cancer risk. Am. J. Epidemiol., in press.
15. Millette, J. R., Clark, P. J., and Pansing, M. F. Exposure to Asbestos from Drinking Water in the United States. Environmental Health Effects Research Report, Office of Research and Development, EPA-600/1–79–028, 1979.
16. Surveillance, Epidemiology, End Results: Incidence and Mortality Data: 1973-1977. NCI Monograph, Vol. 57, National Cancer Institute, 1981.
17. Gordis, S. Should dead cases be matched to dead controls? Am J. Epidemiol. 115: 1–5 (1982).
18. R. L. Polk and Co. 1980 Everett City Directory. Kansas City, 1980.
19. Prentice, R. L. Use of the logistic model in retrospective studies. Biometrics 32: 597–606 (1976).
20. Selikoff, I. J., and Lee, D. H. K. Asbestos and Disease. Academic Press, New York, 1978.

Environmental Health Perspectives
Vol. 53, pp. 61–67, 1983

Observations of Cancer Incidence Surveillance in Duluth, Minnesota

by Eunice E. Sigurdson*

In 1973, amphibole asbestos fibers were discovered in the municipal water supply of Duluth, Minnesota. The entire city population of approximately 100,000 was exposed from the late 1950s through 1976 at levels of 1–65 million fibers per liter of water. Because of previous epidemiologic studies that linked mesothelioma, lung and gastrointestinal cancers to occupational exposure to asbestos, surveillance of cancer incidence in residents of Duluth was initiated to determine the health effect from ingestion of asbestos. The methodology of the Third National Cancer Survey (TNCS) and SEER Program was used. Duluth 1969–1971 rates were compared with TNCS rates for the cities of Minneapolis and St. Paul during 1969–1971; Duluth rates during 1974–1976 are compared with Duluth 1969–1971; Duluth rates during 1979–1980 are compared with Duluth 1969–1971 and with Iowa SEER; and a table of the occurrence of malignant mesothelioma is presented. Statistically significant excesses are observed in several primary sites in Duluth residents. However, lung cancer in Duluth females is the only primary site considered also of biological significance. The mesothelioma incidence rate is no more than expected. This paper also describes the problems of long-term surveillance of exposed populations considered at risk of environment cancer, the need for improved study methodologies and the use of federal records for follow up of exposed individuals.

Introduction

Before presenting recent findings from the surveillance of cancer incidence in Duluth, it seems appropriate to review the history of the situation and the duration and intensity of exposure to amphibole in the city, which at the time of exposure had a population of approximately 100,000.

In 1973, amphibole fibers were discovered in the municipal water supply of Duluth through studies done by the Environmental Protection Agency. A Federal Court ruling indicated that the fibers were a result of a taconite mining company dumping taconite tailings wastes into Lake Superior since 1955 (*1*). In the late 1950s, the mining company, which is located 50 miles northeast of Duluth on the lake, increased the amount dumped to approximately 67,000 tons per day, which continued into 1980. Taconite is low-grade iron ore that is mined and processed into pellets of higher grade iron ore and shipped to steel mills on the Great Lakes. This particular supply of taconite is mined from amphibole-bearing rock. Cummingtonite-grunerite is the principal amphibole in this deposit.

U.S. EPA data on Duluth water samples in 1939–1940 and 1949–1950 indicated trace amounts of fibers, but samples from 1965 contained large amounts of amphibole. It is not known when amphibole fiber levels increased to those levels. The 1973 tapwater samples collected by EPA contained 1 to 30 million amphibole fibers/L, the level generally dependent on lake weather conditions and length of time the water was in the water distribution system (*2*). In 1976, water samples were collected from 20 homes in Duluth and a range of 2 to 64 million amphibole fibers/L was found. Storm conditions in the lake have produced levels as high as 100 million fibers/L. Electron microscope studies at the Minnesota Department of Health have indicated that the physical characteristics of the fibers are: a mean length of 1.13 μm, a mean width of 0.18 μm and thus, an aspect ratio (*length/width*) of 6.5:1 (unpublished, Minnesota Department of Health, 1978). The aspect ratio of Duluth amphibole is double that of the OSHA arbitrary definition of a fiber with a minimum aspect ratio of 3:1.

*Chronic Disease Epidemiology Section, Minnesota Department of Health, 717 S.E. Delaware Street, Minneapolis, MN 55440.

A water filtration plant in Duluth became operational in January 1977, removing 99.9% of the fibers. The length of exposure for Duluth residents may be considered to be 17 years, 1960–1976, and the level of exposure to be in the range of 1 to 65 million fibers/L. Until recently, monitoring of the water supply occurred daily at one of several points in the entire water distribution system.

In 1974, because of the known health risk of cancer to those occupationally exposed by inhalation to asbestos (*3–11*) and the public health concern regarding the unknown risk of those ingesting asbestos from a public water supply, a study of cancer in Duluth residents was designed to determine cancer incidence during 1969–1981.

Generally, 10–20 yr is considered the induction period for cancer of most primary sites and may be as long as 30–50 yr following asbestos exposure (*12*). Therefore, Duluth cancer incidence rates during 1969–1971, approximately 10 yr following the initiation of exposure, are considered baseline rates with which to compare the results of surveillance through time. The rates beginning in the mid-1970s would be the first rates that could reflect any possible increase in cancer due to exposure, assuming exposure began in approximately 1960.

Methods

The methodology used was that of the Third National Cancer Survey (TNCS) of 1969–1971 (*13*) and the current SEER Program at the National Cancer Institute (*14*). Personnel who worked on the Minneapolis-St. Paul component of the TNCS have conducted the study in Duluth. This has been important for the standardization and uniformity of methodology and data collection procedures and for the comparison of data between study areas.

The study requires the identification of all cancer cases in the study population through the review of hospital medical records, pathology and autopsy reports, and death certificates, and the abstracting of patient charts at the three hospitals in Duluth, the Mayo Clinic, and the University of Minnesota and Veterans Administration Hospitals in Minneapolis. The abstracted information includes name and address of patient, age, sex, race, date of diagnosis, primary site and histology, and hospital of diagnosis.

Duluth rates during 1969–1971 are compared with TNCS rates for the cities of Minneapolis and St. Paul, by using the Mantel-Haenszel method for determination of statistical significance (*15*). Minneapolis and St. Paul are considered excellent

Table 1. Characteristics of populations of Duluth and comparison cities.[a]

	Duluth	Minneapolis	St. Paul
Total population (1970 census)	100,578	434,400	309,980
Median age			
Males	27.8	27.8	26.3
Females	31.5	30.7	29.7
Birthplace			
% born in Minnesota	69.9	67.1	72.6
% foreign-born	5.2	4.8	3.9
% foreign stock (foreign-born individuals + natives of foreign or mixed parentage)	27.9	23.9	21.8
Norwegian and Swedish	10.4	9.4	4.4
German and Austrian	2.5	3.4	5.2
Finnish	2.8	0.5	0.2
% white	98.3	95.6	95.4
% who lived in same county in 1965 as in 1970	82.1	75.9	79.7
Median no. of school years completed (for population ≥ 25 yr old)	12.3	12.3	12.2
% of males ≥ 16 yr old in civilian labor force unemployed	6.0	4.2	3.6
% families below poverty level	7.4	7.2	6.4
Median family size	3.48	3.26	3.52
Median family income	$9,313	$9,960	$10,544
% in selected occupations			
Professional, technical, and kindred workers	16.4	16.5	16.5
Craftsmen, foremen, and kindred workers	13.0	10.7	11.7
Laborers except farmers	4.8	4.2	4.3
% in selected industries			
Mining	0.6	0.1	0.1
Construction	4.8	4.2	4.7
Manufacturing	16.4	20.5	25.1

[a]Based on 1970 U.S. census data.

comparison cities because of similar population characteristics. Table 1 lists data from the 1970 census and generally indicates that age, sex and race distributions and socioeconomic, occupational, and ethnic factors (all of which are associated with cancer incidence rates) are similar for the three cities. Important detailed dissimilarities are noted for the Duluth population; namely, smaller size, higher percentage of foreign stock, higher percentage of males 16 years of age and older unemployed, a sixfold excess of the population in mining industries and lower percentage in manufacturing industries. Minneapolis and St. Paul municipal water supplies are also known to have very low levels of amphibole fibers, at least an order of magnitude lower than Duluth (Minnesota Department of Health, unpublished data, 1979).

There are no existing cancer incidence data for Minneapolis and St. Paul since 1969–1971. Standardized morbidity ratios (SMRs) compare Duluth rates during the later years of the current study period of 1969–1981 with rates from the Iowa component of the SEER Program. Tests of statistical significance were performed, using a method defined by Bailar (*16*). Future analysis of the entire study period will include comparison with SEER data from selected cities of Iowa because of similar population characteristics as described above for the cities of Minneapolis and St. Paul. Those Iowa cities are known to be virtually free of amphibole fibers in their municipal water supplies (J. Millette, Health Effects Research Laboratory, U.S. EPA, personal communication).

It is important to note that the methodologies of the cancer incidence studies of Duluth, San Francisco, and Seattle (all presented at this workshop) are virtually identical. A common study methodology makes comparison of those study results quite meaningful among the three cities, all with different exposure factors.

Results

Tables 2 and 3 contain absolute numbers of Duluth cancer cases and average annual age-adjusted incidence rates of selected primary sites for males and females, respectively, in the cities

Table 2. Cancer incidence rates of selected sites, male residents of Duluth and comparison cities 1969–1971, and Duluth 1974–1976.

	Average annual age-adjusted rates per 100,000 population[a]					
	1969–1971				1974–1976, Duluth	
	Duluth		Minneapolis	St. Paul		
Primary site	Rate	Number	rate	rate	Rate	Number
All sites combined	388.0	667	375.7	362.4	361.4	617
Total gastrointestinal	109.6	191	106.8	106.8	94.4	163
Stomach	20.2	36	16.7	14.3	15.0	26
Colon, excluding rectum	34.3	60	40.8	42.6	28.5	49
Transverse colon	6.4	11	6.0	7.9	7.2	12
Descending colon	4.0	7	3.9	3.8	1.6	3
Sigmoid	7.4	13	12.9	15.2*	8.8	15
Cecum	7.9	14	10.6	8.0	7.9	14
Ascending colon	4.9	8	3.8	4.3	1.7	3
Rectum	13.8	24	13.1	17.6	21.6	37
Liver	3.2	5	3.0	2.3	0.6	1
Pancreas	16.9	30	14.2	11.8	14.4	25
Peritoneum, retroperitoneum, intra-abdominal	4.3	7	1.4*	1.5	0	0
Other peritoneum and digestive	2.8	5	0.3†	0.2†	1.1	2
Lung and bronchus	75.5	128	70.1	64.7	68.6	116
Pleura	0	0	0.3	0.4	3.1	5
Prostate	90.4	161	69.3†	68.0†	71.7*	127
Bladder	21.5	37	23.7	20.0	24.2	42
Kidney and renal pelvis	10.8	17	11.6	10.5	8.1	14
Lymphomas	8.0	13	12.4	11.1	14.1	22
Multiple myeloma	5.9	10	4.0	3.8	1.7	3
Leukemias	13.6	23	12.9	15.6	14.8	25

[a]Rates were age-adjusted using the 1970 Minnesota population as the standard.
*Statistically significant difference with Duluth 1969–1971 at $p \leq 0.05$.
†Statistically significant difference with Duluth 1969–1971 at $p \leq 0.01$.

Table 3. Cancer incidence of selected sites, female residents of Duluth and comparison cities 1969–1971, and Duluth 1974–1976.

	Average annual age-adjusted rates per 100,000 population[a]					
	1969–1971				1974–1976, Duluth	
	Duluth		Minneapolis	St. Paul		
Primary site	Rate	Number	rate	rate	Rate	Number
All sites combined	332.1	613	320.2	327.2	333.7	631
Total gastrointestinal	83.8	162	78.8	84.2	74.5	145
Stomach	9.9	19	9.3	11.8	7.8	15
Colon, excluding rectum	34.7	67	37.9	38.7	31.7	62
Transverse colon	7.7	15	5.1	5.7	4.0	8
Descending colon	1.2	2	2.5	4.4	2.0	4
Sigmoid	6.3	12	10.9	11.8*	9.2	18
Cecum	10.1	20	9.7	8.6	9.2	18
Ascending colon	4.8	9	5.7	5.3	5.2	10
Rectum	8.2	16	9.0	9.2	10.0	19
Liver	1.0	2	0.9	2.2	0	0
Pancreas	13.2	26	8.3	9.9	8.7	17
Peritoneum, retroperitoneum, intra-abdominal	0.5	1	1.2	2.6	1.1*	2
Other peritoneum and digestive	1.5	3	0.8	0.2	0.5	1
Lung and bronchus	9.3	18	14.7	12.6	17.9*	34
Pleura	0	0	0.1	0.2	0.5	1
Breast	89.9	163	90.8	78.2	85.4	162
Cervix	25.3	41	18.1	28.3	15.7	25
Corpus	27.1	53	18.9*	24.8	43.5*	84
Bladder	6.3	12	6.8	9.1	7.1	14
Kidney and renal pelvis	8.4	16	6.6	6.5	7.3	14
Lymphomas	6.1	11	9.2	10.6	11.8	22
Multiple myeloma	0.5	1	6.2†	3.1	4.2*	8
Leukemias	10.6	19	10.0	7.8	9.8	18

[a]Rates were age-adjusted using the 1970 Minnesota population as the standard.
*Statistically significant difference with Duluth 1969–1971 at $p \leq 0.05$.
†Statistically significant difference with Duluth 1969–1971 at $p \leq 0.01$.

of Duluth, Minneapolis, and St. Paul during 1969–1971 and Duluth during 1974–1976. Duluth rates during the earlier time period are compared with Minneapolis and St. Paul, and Duluth rates during the later period are compared with rates during the earlier period. Comparisons have been made for 84 primary sites, and those that may be considered at risk of cancer from ingestion of asbestos are presented in the tables.

In the 1969–1971 comparisons, excesses in Duluth that are statistically significant are observed, by sex and primary site, for male peritoneum, retroperitoneum and intra-abdominal; male other peritoneum and digestive; female uterine corpus; and male prostate. In the comparison between the two Duluth time periods, statistically significant increases are observed for female peritoneum, retroperitoneum, and intra-abdominal; female lung cancer; female uterine corpus; and female multiple myeloma. A decrease in male prostate is statistically significant. Data from some of these sites involve very small numbers and must be interpreted with caution even though statistically significant.

Table 4 provides the most recent data for Duluth, for 1979–1980 for males and females separately, again for selected sites. Absolute numbers of cases, average annual crude incidence rates, and SMRs compare Duluth 1979–1980 rates with rates for Duluth 1969–1971 and with the State of Iowa SEER 1973–1977 for whites only. The only statistically significant excesses are Duluth female lung cancer during 1979–1980 in comparison with Duluth rates during 1969–1971, and Duluth male stomach cancer during 1979–1980 in comparison with Iowa SEER 1973–1977. However, the Duluth female lung cancer rate is not statistically significant when compared with that for Iowa, and the Duluth male stomach cancer rate is not statistically significant when compared with that for Duluth 1969–1971.

Table 5 contains the number of cases of pleural and peritoneal malignant mesothelioma in residents of Duluth, by year of diagnosis during

Table 4. Duluth cancer incidence for selected sites during 1979–1980, and standard morbidity ratios.

Primary site	Total number		Duluth average annual crude incidence rate 1979–1980[a]		SMR, Duluth 1979–1980[b]		SMR, Duluth 1979–1980[c]	
	Male	Female	Male	Female	Male	Female	Male	Female
Stomach	18	13	20.5	13.3	0.8	0.9	1.7†	1.6
Colon, excluding rectum	44	50	50.1	51.1	1.1	1.0	1.2	0.9
Rectum and rectosigmoid	19	14	21.6	14.3	1.1	0.7	0.9	0.7
Liver	2	3	4.6	3.1	0.8	1.5	1.0	1.7
Pancreas	6	11	6.8	11.2	0.3	0.6	0.5	0.9
Peritoneum, retroperitoneum and intra-abdominal	1	2	—	—	—	—	—	—
Total gastrointestinal	90	93	102.6	95.0	0.8	0.8	1.0	0.9
Lung and bronchus	64	29	72.9	29.6	0.8	2.3*	0.9	1.5
Pleura	1	0	—	—	—	—	—	—
Prostate	81	—	92.3	—	0.8	—	1.2	—
Female breast	—	93	—	95.0	—	0.8	—	0.9
Bladder	28	11	31.9	11.2	1.2	1.2	1.1	1.0
Kidney and renal pelvis	10	5	11.4	5.1	0.9	0.4	1.2	0.8
All sites combined	364	399	414.8	407.7	0.9	0.9	1.0	0.9

[a]Based on the Duluth 1980 population.
[b]Expected numbers of cancers from application of 1969-1971 Duluth age-sex-specific incidence rates to the 1980 Duluth population.
[c]Expected numbers of cancers from application of the 1973-1977 Iowa white age-sex-specific incidence rates to the 1980 Duluth population.
*Statistically significant at $p < 0.01$.
†Statistically significant at $p < 0.05$.

1969–1980. There were nine cases of pleural and one case of peritoneal during the 12 years of observation. For such a rare disease, it is appropriate to average the number of cases during the study period. This results in an average of 0.75 cases per 100,000 population of pleural mesothelioma occurring per year compared to the observed frequency of 0.2 cases per 100,000 per year in the Minneapolis-St. Paul component of the TNCS. The SMR (9 observed cases, 2.4 expected) of 3.75 is statistically significant at $p = 0.01$ (*16*). An increase in diagnostic surveillance during the mid-1970s, generated from increased concern in this community relative to malignant mesothelioma, may partially account for these differences. Based on the observed frequency of pleural malignant mesothelioma, one would expect approximately one newly diagnosed case per year. In fact, six cases were ascertained during the 3-yr period of heightened concern, 1974–1976, which is double the expected frequency of three.

Table 5. Malignant mesothelioma in residents of Duluth 1969–1980.

	Pleura		Peritoneum	
	Male	Female	Male	Female
1969	—	—	—	—
1970	—	—	—	—
1971	—	—	—	—
1972	—	—	—	—
1973	1	—	—	—
1974	2	1	—	—
1975	1	—	—	—
1976	—	2	—	1
1977	—	—	—	—
1978	1	—	—	—
1979	—	—	—	—
1980	1	—	—	—
Total	6	3	—	1

Discussion

We are unable to explain the variations in rates and the differences that are statistically significant. However, it is clear that rate differences between geographic areas and through time do occur without any apparent biologic reason. Also, in the determination of statistical significance at the $p = 0.05$ level, for every 100 comparisons, 5% will be statistically significant by chance alone. In our opinion, the only statistically significant result with clear biological significance is the increase in female lung cancer in Duluth, undoubtedly a reflection of the increase in cigarette smoking over the past few decades. This increasing trend of lung cancer in women is seen nationally (*14*). The marginally statistically significant excess of stomach cancer in Duluth males, compared with Iowa, is consistent with historical observations of stomach cancer mortality for St. Louis County, the location of Duluth (*17*). The

lack of a statistically significant excess in Duluth females, compared with Iowa, is probably due to the smaller number of female cases. Historically, the rates have been high for stomach cancer in males and females long before the occurrence of asbestos exposure. However, the impact of asbestos exposure on the risk of stomach cancer in excess of the risk attributed to known risk factors (i.e., Scandinavian dietary practices) on current cases, cannot be determined (*18*). Other differences of Duluth cancer incidence rates may be important, and a longer time of surveillance coupled with evaluation of mobility and follow-up of the exposed population will need to be conducted before these issues can be resolved.

Needs of Further Research

In my opinion, it will be important to complete the analysis of study period 1969–1981 so that three solid years of data during 1979–1981 around the 1980 census year can be analyzed. At that time, thorough review and analyses of all the data should be conducted. In addition, plans should be made to continue the surveillance, possibly using a modified methodology to accommodate the entire length of exposure of 17 yr and the induction periods of 30–50 yr. Furthermore, the intent of the study design was to incorporate methods for determining length of residency of Duluth cancer cases as a measure of length of exposure and also for determining migration patterns of the exposed population more scientifically than the Census Bureau data currently can permit. A basic problem in study methodology arises in such long-term surveillance when one questions to what degree the observed cancer incidence is measuring the occurrence of disease in the actual exposed population, even for the city of Duluth, which is considered to be a very stable population.

Similar questions also arise for this and other diseases perceived to be environmental threats to human health and yet having very long latent periods. These questions require new methodologies and procedures such as rapidly registering exposed individuals at time of exposure, taking biologic specimens, and incorporating methods of long-term follow-up. Such follow-up would be accomplished much more easily, and scientific study would probably be much more valid if it were possible to use some IRS records and Census Bureau records of individuals in large, exposed populations. Use of those records would greatly enhance and enable long-term follow-up in epidemiologic and biomedical research and would reduce the expense of more difficult and time-consuming follow-up procedures, which are now required (*19*).

It is apparent that there is another major area of needed epidemiologic research regarding the health effects from the ingestion of asbestos in drinking water—the need for development of methods for clinical laboratory studies applied within an epidemiologic design, which would address questions related to low-level dose-response effects, host sensitivity and reactivity for large populations, and the effects of three variables known to affect carcinogenicity of asbestos fibers: the structure, geometry, and surface adsorption characteristics of fibers. Currently, it is difficult to predict the carcinogenic behavior of fibers *in vivo* in both animals and humans.

The author gratefully acknowledges the assistance of Barry Levy, M.D., Jack Mandel, Ph.D., Richard McHugh, Ph.D.; Alan Bender, D.V.M., Ph.D., for assistance with the design of the project and review of manuscripts; Helen Jagger, R.N., M.P.H., and Hazel Gallup, R.N., for data collection and field work; Ethelyn Yliniemi for typing of manuscripts; also the hospital administrators, record room administrators and staffs in Duluth hospitals and Leonard Kurland, M.D., at Mayo Clinic, all of whom have been very supportive of the study. A special thanks to James Stebbings, Sc.D., Kyle Bishop, and Leonard M. Schuman, M.D.

The Environmental Protection Agency provided the Minnesota Department of Health with a 1-yr grant just to study the feasibility of conducting this long term follow-up study. It was hoped that the principal investigator, Dr. Leonard J. Michienzi, would have developed a plan for implementing the follow-up that is now called for in this report. Funding for the study reported on here was provided by the Environmental Protection Agency starting in December 1977 and continued through April 1982 as grant R805428.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. United States of America vs. Reserve Mining Company, 5-72 Civil 19.
2. Cook, P. M., Glass, G. E., and Tucker, J. H. Asbestiform amphibole materials: detection and measurement of high concentrations in municipal water supplies. Science 185: 853–855 (1974).
3. Selikoff, I. J., Churg, J., and Hammond, E. C. Asbestos exposure and neoplasia. J. Am. Med. Assoc. 188: 22–26 (1964).
4. Enterline, P. E. Mortality among asbestos products workers in the United States. Ann. N.Y. Acad. Sci. 132: 156–165 (1965).
5. McDonald, J. C., McDonald, A. D., and Gibbs, G. W. Mortality in the chrysotile asbestos mines and mills of Quebec. Arch. Environ. Health 22: 677–686 (1971).
6. Enterline, P., Decoufle, P., and Henderson, V. Mortality in

relation to occupational exposure in the asbestos industry. J. Occup. Med. 14: 897–903 (1972).
7. Selikoff, I. J., Hammond, E. C., and Churg, J. Carcinogenicity of amosite asbestos. Arch. Environ. Health 25: 183–186 (1972).
8. Elmes, P. C., and Simpson, M. J. C. Insulation workers in Belfast. 3. Mortality 1940–1966. Brit. J. Ind. Med. 28: 226–236 (1971).
9. Hammond, E. C., Selikoff, I. J., and Churg, J. Neoplasia among insulation workers in the United States with special reference to intra-abdominal neoplasia. Ann. N. Y. Acad. Sci. 132: 519–525 (1965).
10. Mancusso, T. F. Asbestos and neoplasia: epidemiology: discussion. Ann. N.Y. Acad. Sci. 132: 589–594 (1965).
11. Selikoff, I. J., Hammond, E. C., and Seidman, H. Cancer risk of insulation workers in the United States. Insulation Hyg. Prog. Repts. 6: 1–8 (1974).
12. Selikoff, I. J., and Lee, D. H. Asbestos and Disease. Academic Press, New York, 1978.
13. National Institutes of Health. Third National Cancer Survey: Incidence Data. National Cancer Institute Monograph 41, HEW Publication No. NIH 75-787, 1975.
14. National Institutes of Health. Surveillance, Epidemiology, End Results, Incidence and Mortality Data: 1973-77. National Cancer Institute Monograph 57, NIH Publication No. 81-2330, 1981.
15. Mantel, N. Chi-square tests with one degree of freedom. J. Am. Stat. Assoc. 58: 690–699 (1973).
16. Bailar, J. C., and Ederer, F. Significance factors for the ratio of Poisson variable to its expectations. Biometrics 20: 639–643 (1964).
17. Mason, T., and McKay F. Atlas of Cancer Mortality for U.S. Counties: 1950–1969. DHEW Publ. No. (NIH) 75-780, 1975.
18. Nomura, A. Stomach. In: Cancer Epidemiology and Prevention (D. Schottenfeld and J. Fraumeni, Eds.), W. B. Saunders, Philadelphia, 1982, pp. 624–637.
19. Sigurdson, E. Description and comparison of two population-based studies of Duluth, Minnesota, as a follow-up of asbestos exposure and recommendations for facilitating similar studies in the future. J. Environ. Sci. Health A17: 463–471 (1982).

Environmental Health Perspectives
Vol. 53, pp. 69–78, 1983

Effect of Population Density on the Results of the Study of Water Supplies in Five California Counties

by Paul M. Conforti*

Two previous studies (1969–1971 and 1969–1974) examined the association between cancer incidence and chrysotile asbestos ingested through drinking water in the San Francisco–Oakland Standard Metropolitan Statistical Area (SMSA). Population density, an important covariable in the association between cancer and environmental agents, was not included in the analyses of these studies. The present work determines the effect of this covariable on the results of the second San Francisco–Oakland SMSA study. The original and reanalyzed results are compared to reassess the association between cancer and asbestos. The only change in the regression procedures of the original studies was the addition of population density as an independent variable in the reanalysis. The results of the reanalysis showed that population density had little effect on the results of the second study. Slightly more significance was found for asbestos regression coefficients in the reanalysis, including population density, than in the original analysis. These regression coefficients for asbestos indicated a positive association between ingested chrysotile asbestos and some cancer body sites. The conclusion of the reanalysis was that population density was distributed across the San Francisco–Oakland SMSA in such a way that it had little effect on the observation of an association between ingested asbestos and cancer.

Introduction

The association between ingested chrysotile asbestos and cancer was investigated in a project entitled "Asbestos in Domestic Water Supplies in Five California Counties," which was sponsored by the U.S. Environmental Protection Agency (U.S. EPA). In that project, standard incidence ratios for cancers of various body sites were analyzed for their associations with ingested asbestos through drinking water while the covariables of socioeconomic status, marital status, and asbestos-related industries were controlled. The study area was the San Francisco–Oakland Standard Metropolitan Statistical Area (SMSA) and the unit of observation was the census tract. Questions have been raised concerning the validity of the results of that work because population density of the SMSA and census tracts were not considered in the analysis. The present work includes population density in a reanalysis of the data accumulated under the U.S. EPA-Sponsored project.

Two studies emerged from the research on asbestos and cancer in the San Francisco–Oakland SMSA. Cancer incidence between 1969 and 1971 was analyzed in the initial study and has been reported by Kanarek et al. (*1*). A second study of cancer incidence between 1969 and 1974 was carried out and published by Conforti et al. (*2*). The present work is a reanalysis of the 6-yr data base (1969–1974) to determine the effect of population density on the observation of an association between ingested asbestos and cancer.

Methodology

Population density data (square kilometers of each 1970 census tract and population figures) were obtained from the Socio-Economic-Environmental Demographic Information System (SEEDIS) of the Computer Science and Mathe-

*Group in Biostatistics, 140 Earl Warren Hall, University of California at Berkeley, Berkeley, CA 94720.

matics Department, Lawrence Berkeley Laboratory, University of California, Berkeley (*3*). The square kilometers for each 1970 census tract were calculated in the SEEDIS system from coordinates of latitude and longitude on the boundaries of the census tracts. Coordinates were used to create triangles within the tracts, and the areas of these triangles were calculated and summed to yield the square kilometers per census tract. The coordinates were obtained from the Dual Independent Map Encoding (DIME) file of the 1970 Census of Population and Housing of the Bureau of the Census (*4*).

Since the reanalysis was made for the 6-yr cancer incidence data (1969–1974) and the midpoint of that study period was January 1, 1972, extrapolated population figures (based on 1960 and 1970 censuses) for January 1, 1972 were used in calculating population density. The extrapolation required identical boundaries between 1960

Table 1. Cancer sites analyzed in asbestos study of San Francisco–Oakland SMSA, incidence, 1969–74.

Site no.	Name	RCE site no.[a]	ICD 8th Rev. site nos.[b]
1	All sites	010–940	
2	All digestive	500–590	150–159
3	Digestive tract	500–549	150–154
4	Esophagus	500–509	150
5	Stomach	510–519	151
6	Small intestine	520–529	152
7	Colon	530–539	153
8	Rectum	540–549	154
9	Digestive-related organs	550–589	155–158
10	Liver	550–551	155
11	Gallbladder	560–569	156
12	Pancreas	570–579	157
13	Retroperitoneum	580–589	158
14	Digestive organ NOS[c]	590	159
15	All respiratory	619–639	161–163
16	Larynx	619	161
17	Trachea, bronchus, lung	620–621	162
18	Pleura	630	163.0
19	Mediastinum	631	163.1
20	Breast	700–709	174
21	Female reproductive	710–759	180–183
22	Cervix uteri	710	180
23	Corpus uteri	720	182.0
24	Ovary	750	183.0
25	Prostate	770	185
26	Urinary	800–810	188–189
27	Kidney	800–804	189
28	Bladder	810	188
29	Brain	930	191
30	Thyroid	940	193
31	Hodgkin's disease	010	201
32	Leukemia	040–049	204–208
33	Lung small cell carcinoma	621 RCE-hist 804	
34	Lung squamous	621 RCE-hist 807	
35	Lung adenocarcinoma NOS[c]	621 RCE-hist 814	

[a]Manual of Tumor Nomenclature and Coding.
[b]Eighth Revision International Classification of Diseases, 1968.
[c]Not otherwise specified.

Table 2. Number of super tracts in tract groupings by chrysotile asbestos fiber counts of the San Francisco–Oakland SMSA, 1972.

	Chrysotile asbestos fibers/L				
	25,000–305,812	310,000–330,000	350,000–19,542,843	20,000,000–36,000,000	Total
Number of super tracts	92	110	107	101	410

Table 3. Frequency distribution of population density for 427 super tracts of the San Francisco–Oakland SMSA, 1972.

Population/km²	Frequency	Relative frequency, %	Cumulative frequency, %
0–1000	82	19.2	19.2
1000–2000	37	8.7	27.9
2000–3000	56	13.1	41.0
3000–4000	63	14.8	55.8
4000–5000	48	11.2	67.0
5000–6000	31	7.3	74.3
6000–7000	20	4.7	79.0
7000–8000	9	2.1	81.1
8000–9000	14	3.3	84.4
9000–10000	9	2.1	86.5
10000–11000	8	1.9	88.4
11000–12000	8	1.9	90.3
12000–13000	8	1.9	92.2
13000–14000	3	0.7	92.9
14000–15000	5	1.2	94.1
15000–16000	3	0.7	94.8
16000–17000	1	0.2	95.0
17000–18000	1	0.2	95.2
18000–19000	3	0.7	95.9
19000–20000	2	0.5	96.4
20000–21000	1	0.2	96.6
21000–22000	0	0.0	96.6
22000–23000	2	0.5	97.1
23000–24000	2	0.5	97.6
24000–25000	0	0.0	97.6
25000–26000	3	0.7	98.3
26000–27000	1	0.2	98.5
27000–28000	0	0.0	98.5
28000–29000	2	0.5	99.0
29000–30000	0	0.0	99.0
30000–31000	0	0.0	99.0
31000–32000	0	0.0	99.0
32000–33000	2	0.5	99.5
33000–34000	1	0.2	99.7
34000–35000	0	0.0	99.7
35000–36000	0	0.0	99.7
36000–37000	0	0.0	99.7
37000–38000	1	0.2	99.9
38000–39000	0	0.0	99.9
39000–40000	1	0.2	100.0
	427		

and 1970 census tracts. Census tracts in 1960 and 1970 were not in one-to-one correspondence. Census tract groupings (super tracts) were developed that had corresponding geographical boundaries between censuses. In 1970 there were 722 census tracts in the San Francisco–Oakland SMSA. The grouping of census tracts into super tracts yielded 427 super tracts. Square kilometers for each of the 722 census tracts were summed in each of their corresponding super tracts to yield square kilometers per super tract. Population density was then calculated for each super tract by dividing population by square kilometers to yield population per square kilometer for each super tract.

Standard incidence ratios for 35 body sites of cancer were used as the dependent variables in the analyses. The independent variables were socioeconomic status, marital status, asbestos-related industries, asbestos in drinking water, and population density. Socioeconomic status was

Table 4. Frequency distribution of population density for 199 super tracts of the West Bay Area, 1972.

Population/km²	Frequency	Relative frequency, %	Cumulative frequency, %
0–1000	35	17.6	17.6
1000–2000	14	7.0	24.6
2000–3000	21	10.6	35.2
3000–4000	24	12.1	47.3
4000–5000	13	6.5	53.8
5000–6000	10	5.0	58.8
6000–7000	5	2.5	61.3
7000–8000	5	2.5	63.8
8000–9000	11	5.5	69.3
9000–10000	6	3.0	72.3
10000–11000	8	4.0	76.3
11000–12000	7	3.5	79.8
12000–13000	7	3.5	83.3
13000–14000	3	1.5	84.8
14000–15000	4	2.0	86.8
15000–16000	3	1.5	88.3
16000–17000	1	0.5	88.8
17000–18000	1	0.5	89.3
18000–19000	3	1.5	90.8
19000–20000	2	1.0	91.8
20000–21000	1	0.5	92.3
21000–22000	0	0.0	92.3
22000–23000	2	1.0	93.3
23000–24000	2	1.0	94.3
24000–25000	0	0.0	94.3
25000–26000	3	1.5	95.8
26000–27000	1	0.5	96.3
27000–28000	0	0.0	96.3
28000–29000	2	1.0	97.3
29000–30000	0	0.0	97.3
30000–31000	0	0.0	97.3
31000–32000	0	0.0	97.3
32000–33000	2	1.0	98.3
33000–34000	1	0.5	98.8
34000–35000	0	0.0	98.8
35000–36000	0	0.0	98.8
36000–37000	0	0.0	98.8
37000–38000	1	0.5	99.3
38000–39000	0	0.0	99.3
39000–40000	1	0.5	100.0
	199		

measured by median family income and mean years of schooling. Marital status was computed as the proportion of unmarried persons in the population. Asbestos-related industries were calculated as the proportion of workers with potential occupational exposure to asbestos in industries such as the construction, electrical, and textile industries. Asbestos was measured as the number of chrysotile fibers per liter of drinking water. These variables were recorded for each super tract of the San Francisco–Oakland SMSA.

Regression analyses were performed including population density. Correlations were calculated between standard incidence ratios of cancer and population density. Descriptive and summary statistics were also computed.

Results

The cancer sites analyzed and their sources are presented in Table 1. All major cancers except skin and bone are included. Some of the cancer

Table 5. Frequency distribution of population density for 228 super tracts of the East Bay Area, 1972.

Population/km²	Frequency	Relative frequency, %	Cumulative frequency, %
0– 1000	47	20.6	20.6
1000– 2000	23	10.1	30.7
2000– 3000	35	15.4	46.1
3000– 4000	39	17.1	63.2
4000– 5000	35	15.4	78.6
5000– 6000	21	9.2	87.8
6000– 7000	15	6.6	94.4
7000– 8000	4	1.8	96.2
8000– 9000	3	1.3	97.5
9000–10000	3	1.3	98.8
10000–11000	0	0.0	98.8
11000–12000	1	0.4	99.2
12000–13000	1	0.4	99.6
13000–14000	0	0.0	99.6
14000–15000	1	0.4	100.0
	228		

sites listed are individual body sites and others are cancer site groupings such as Site No. 1 (all sites) and Site No. 2 (all digestive). Individual and grouped body sites are apparent from their Manual of Tumor Nomenclature and Coding code numbers and their Eighth Revision International Classification of Diseases code numbers.

Table 2 presents the distribution of super tracts grouped into four intervals of asbestos. The table includes 410 super tracts instead of the original 427 because 17 super tracts with incomplete information were removed from the analysis. In general, the two lowest asbestos intervals contain the super tracts of the two counties east of the San Francisco Bay (Alameda and Contra Costa), and the two highest asbestos intervals contain the super tracts of the three counties west of San Francisco Bay (San Francisco, San Mateo, and Marin).

Tables 3–10 show the frequency distributions of population density by 1000 population per square kilometer for super tracts of the SMSA, West Bay and East Bay Counties, and San Francisco, San Mateo, Marin, Alameda, and Contra Costa Counties. For the SMSA, the majority of super tracts had populations per square kilometer of less than 5000. Of the 427 super tracts, 86% had less than 10,000 population/km². The mean population per square kilometer for the SMSA was 5391.38, with a standard deviation of 6044.29.

West Bay and East Bay frequency distributions (Tables 4 and 5, respectively) showed that the most dense super tracts—those above 15,000 population per square kilometer—were found in the West Bay Counties. The county frequency distributions of population density (Tables 6–10) indicated that the West Bay area had one high-density county (San Francisco) and two low-density counties (San Mateo and Marin). The East Bay area had one moderately dense county (Alameda) and one low-density county (Contra Costa).

Table 11 presents the correlation coefficients of cancer with population density for the 35 cancer sites analyzed by sex for the white study population. For the males, of 31 cancer sites (4 of the listed sites are for female reproductive cancers), 15 had positive correlation coefficients and 16 had negative coefficients. Three of the 15 positive coefficients were significant ($p < 0.05$), whereas 10 of the 16 negative coefficients were significant. This may be a bit misleading, however. Note that Site No. 1 (all sites) is significantly and positively associated with population density. This is also true for Site No. 15 (all respiratory). Although in general it appeared that cancer was negatively associated with population density for males, closer scrutiny of this table indicated that a positive association existed between most major cancers and population density.

For females, the pattern is markedly different. Of 34 cancer sites (one of the 35 sites is for male reproductive cancer), 3 were positive and 31 were negative. None of the positive coefficients was significant, but 17 of the negative coefficients were significant. The major cancers (digestive, respiratory, breast, and female reproductive) were negative and significant. The correlation of asbestos with population density was 0.486.

The regression of cancer standard incidence ratios on asbestos, covariables, and population

Table 6. Frequency distribution of population density for 103 super tracts of San Francisco County, 1972.

Population/km²	Frequency	Relative frequency, %	Cumulative frequency, %
0– 1000	2	1.9	1.9
1000– 2000	1	1.0	2.9
2000– 3000	2	1.9	4.8
3000– 4000	4	3.9	8.7
4000– 5000	6	5.8	14.5
5000– 6000	9	8.7	23.2
6000– 7000	4	3.9	27.1
7000– 8000	4	3.9	31.0
8000– 9000	11	10.7	41.7
9000–10000	6	5.8	47.5
10000–11000	8	7.8	55.3
11000–12000	7	6.8	62.1
12000–13000	7	6.8	68.9
13000–14000	3	2.9	71.8
14000–15000	4	3.9	75.7
15000–16000	3	2.9	78.6
16000–17000	1	1.0	79.6
17000–18000	1	1.0	80.6
18000–19000	3	2.9	83.5
19000–20000	2	1.9	85.4
20000–21000	1	1.0	86.4
21000–22000	0	0.0	86.4
22000–23000	2	1.9	88.3
23000–24000	2	1.9	90.2
24000–25000	0	0.0	90.2
25000–26000	3	2.9	93.1
26000–27000	1	1.0	94.1
27000–28000	0	0.0	94.1
28000–29000	2	1.9	96.0
29000–30000	0	0.0	96.0
30000–31000	0	0.0	96.0
31000–32000	0	0.0	96.0
32000–33000	1	1.0	97.0
33000–34000	1	1.0	98.0
34000–35000	0	0.0	98.0
35000–36000	0	0.0	98.0
36000–37000	0	0.0	98.0
37000–38000	1	1.0	99.0
38000–39000	0	0.0	99.0
39000–40000	1	1.0	100.0
	103		

density was performed with a natural logarithm transformation of the cancer standard incidence ratios. To avoid taking the natural logarithm of zero, which would result from super tracts with zero observed cancer cases of a particular site, a small constant (0.01) was added to the numerators and denominators of the standard incidence ratios.

Table 12 shows the cancer sites with significant ($p < 0.05$) positive asbestos regression coefficients. Also presented are the p values for these coefficients and the p values for population density coefficients associated with these equations. The p values are based on the t-test for the hypothesis that the population regression coefficients are zero.

For males, the significant cancer sites included Site No. 1 (all sites), most of the digestive and digestive-related cancers, prostate and lung small cell carcinoma. None of the population density coefficients for these equations was significant. For females, the significant cancer sites included

Table 7. Frequency distribution of population density for 72 super tracts of San Mateo County, 1972.

Population/km²	Frequency	Relative frequency, %	Cumulative frequency, %
0–1000	16	22.2	22.2
1000–2000	9	12.5	34.7
2000–3000	17	23.6	58.3
3000–4000	20	27.8	86.1
4000–5000	7	9.7	95.8
5000–6000	1	1.4	97.2
6000–7000	1	1.4	98.6
7000–8000	1	1.4	100.0
	72		

Table 8. Frequency distribution of population density for 24 super tracts of Marin County, 1972.

Population/km²	Frequency	Relative frequency, %	Cumulative frequency, %
0–1000	17	70.8	70.8
1000–2000	4	16.7	87.5
2000–3000	2	8.3	95.8
3000–4000	1	4.2	100.0
	24		

Table 9. Frequency distribution of population density for 166 super tracts of Alameda County, 1972.

Population/km²	Frequency	Relative frequency, %	Cumulative frequency, %
0– 1000	22	13.3	13.3
1000– 2000	14	8.4	21.7
2000– 3000	27	16.3	38.0
3000– 4000	26	15.7	53.7
4000– 5000	28	16.9	70.6
5000– 6000	21	12.7	83.3
6000– 7000	15	9.0	92.3
7000– 8000	4	2.4	94.7
8000– 9000	3	1.8	96.5
9000–10000	3	1.8	98.3
10000–11000	0	0.0	98.3
11000–12000	1	0.6	98.9
12000–13000	1	0.6	99.5
13000–14000	0	0.0	99.5
14000–15000	1	0.6	100.0
	166		

Site No. 1 (all sites) most of the digestive and digestive-related cancers, respiratory and breast cancers. Site Nos. 1, 2, and 3 showed significant negative population density regression coefficients. This corresponds to the highly significant negative correlation coefficients for these sites.

Finally, Table 13 presents a comparison between the regression results of the original study and the reanalysis. The table shows cancer sites with significant ($p < 0.05$) positive asbestos regression coefficients in either study. The cancer sites that were found to have significant positive regression coefficients in the original study were essentially the same ones found to be significant in the reanalysis. For males, the significant sites were the same in both analyses. For females, two extra sites were found in the reanalysis that were not significant in the original study. These were Site Nos. 3 (digestive tract) and 8 (rectum). For males, the levels of significance were almost identical between analyses. The presence of population density in the reanalysis appeared to slightly decrease the levels of significance of the asbestos coefficients in the equations for these sites. For

Table 10. Frequency distribution of population density for 62 super tracts of Contra Costa County, 1972.

Population/km²	Frequency	Relative frequency, %	Cumulative frequency, %
0–1000	25	40.3	40.3
1000–2000	9	14.5	54.8
2000–3000	8	12.9	67.7
3000–4000	13	20.9	88.6
4000–5000	7	11.4	100.0
	62		

Table 11. Correlation coefficients of cancer by population density by site, sex for white population, San Francisco–Oakland SMSA, 1969–1974.

Site no.	Name	White male: Correlation coefficient	White male: p value[a]	White female: Correlation coefficient	White female: p value[a]
1	All sites	0.104	<0.025	–0.192	<0.0001
2	All digestive	0.051		–0.164	<0.001
3	Digestive tract	0.070		–0.216	<0.00001
4	Esophagus	0.038		0.056	
5	Stomach	0.113	<0.025	–0.043	
6	Small intestine	–0.033		–0.114	<0.025
7	Colon	0.073		–0.175	<0.001
8	Rectum	0.032		–0.099	<0.025
9	Digestive-related organs	0.052		–0.019	
10	Liver	0.018		–0.106	<0.025
11	Gallbladder	–0.039		–0.004	
12	Pancreas	–0.073		0.031	
13	Retroperitoneum	–0.085	<0.05	–0.035	
14	Digestive organ NOS[b]	–0.025		–0.063	
15	All respiratory	0.095	<0.05	–0.087	<0.05
16	Larynx	0.031		–0.078	
17	Trachea, bronchus, lung	0.079		–0.080	
18	Pleura	–0.048		0.044	
19	Mediastinum	0.063		–0.079	
20	Breast	–0.052		–0.180	<0.001
21	Female reproductive	—		–0.172	<0.001
22	Cervix uteri	—		–0.035	
23	Corpus uteri	—		–0.249	<0.00001
24	Ovary	—		–0.064	
25	Prostate	0.001		—	
26	Urinary	–0.113	<0.025	–0.137	<0.005
27	Kidney	–0.119	<0.025	–0.077	
28	Bladder	–0.149	<0.005	–0.122	<0.025
29	Brain	–0.142	<0.005	–0.137	<0.005
30	Thyroid	–0.094	<0.05	–0.038	
31	Hodgkin's disease	–0.103	<0.025	–0.112	<0.025
32	Leukemia	–0.107	<0.025	–0.033	
33	Lung small cell carcinoma	–0.099	<0.025	–0.113	<0.025
34	Lung squamous	0.072		–0.008	
35	Lung adenocarcinoma NOS[b]	–0.132	<0.005	–0.118	<0.01

[a]p-value based on t-test for $H: r = 0$, $n = 410$.
[b]Not otherwise specified.

females, the coefficients were generally more significant with population density included in the analysis. In particular, Site Nos. 1 and 2 were much more significant in the reanalysis than in the original analysis.

Conclusions

The inclusion of population density in the analysis of ingested asbestos and cancer had little effect on the results. Therefore, the conclusion is

Table 12. Cancer sites with significant ($p < 0.05$), positive asbestos regression coefficients, associated population density significance, by sex, San Francisco–Oakland SMSA, 1969–1974.

Site no.	Race and sex	Name	Asbestos *p* value	Population density *p* value
	White male			
1	"	All sites	0.046	0.702[a]
2	"	All digestive	0.001	0.764
3	"	Digestive tract	0.007	0.234
4	"	Esophagus	0.003	0.753[a]
5	"	Stomach	0.008	0.151
7	"	Colon	0.021	0.632
9	"	Digestive-related organs	0.001	0.120[a]
12	"	Pancreas	0.001	0.125[a]
25	"	Prostate	0.039	0.686
33	"	Lung small cell carcinoma	0.015	0.067[a]
	White female			
1	"	All sites	0.002	0.001[a]
2	"	All digestive	0.001	0.001[a]
3	"	Digestive tract	0.002	0.001[a]
4	"	Esophagus	0.004	0.306[a]
4	"	Stomach	0.001	0.178[a]
8	"	Rectum	0.044	0.443[a]
9	"	Digestive-related organs	0.004	0.648[a]
12	"	Pancreas	0.002	0.463
13	"	Retroperitoneum	0.014	0.093[a]
15	"	All respiratory	0.001	0.552
17	"	Trachea, bronchus, lung	0.001	0.384
18	"	Pleura	0.035	0.919
20	"	Breast	0.002	0.117[a]

[a]Indicates negative regression coefficient.

that a positive association between ingested asbestos and cancer existed in the San Francisco–Oakland SMSA for 1969–1974 for certain cancer sites. In spite of the persistent relationships across analyses, there are a number of warnings concerning the results and conclusions. The first is that the associations observed in these analyses are indirect, i.e., the units of observation were census tract groupings (super tracts), which means that groups of individuals were aggregated geographically and analyzed as groups. Therefore, the variables measured are applied to the groups. Individual measurements are not made, and the group measurements are assumed to apply to all members of an observational unit. Although the indirect method of analysis is an inexpensive design for a first look at an epidemiologic hypothesis, the underlying assumptions of the method make definitive conclusions untenable. In the asbestos-cancer analyses, the results indicate a possible direction for further research using other, direct method, designs. For instance, digestive cancers in both males and females were highly, significantly, positively associated with ingested asbestos. A case-control study or follow-up study might investigate this particular association.

The second caution is that the number of cancers is small for a number of the body sites studied. For Site No. 1 (all sites), there were approximately 56,000 incidence cases for all ages and both sexes yielding about 130 cases per super tract. For site No. 6 (small intestine), there were fewer than 200 cases for both sexes. Therefore, there were fewer than 0.5 cases per super tract. In other words, there were a large number of super tracts with no cases of cancer of the small intestine. This makes any form of analysis much less powerful in terms of detecting an association.

Another warning about these results is that the 35 body sites analyzed were not independent. Some of the sites were individual body sites for cancer such as Site Nos. 4 (esophagus), 5 (stomach) and 6 (small intestine). Others were body site groupings such as Site Nos. 1 (all sites), 2 (all digestive) and 15 (all respiratory). The most meaningful results are found for the grouped body sites, since they include large numbers of cases and therefore avoid the aforementioned problem of too few cases per super tract. Unfortu-

Table 13. Comparison of significance ($p < 0.05$) for asbestos regression coefficients in 1969–1974 incidence study and reanalysis including population density, San Francisco–Oakland SMSA, 1969–1974.

Site no.	Race and Sex	Name	Original asbestos *p* value	Reanalysis asbestos *p* value
	White male			
1	"	All sites	0.05	0.046
2	"	All digestive	0.001	0.001
3	"	Digestive tract	0.002	0.007
4	"	Esophagus	0.003	0.003
5	"	Stomach	0.002	0.008
7	"	Colon	0.011	0.021
9	"	Digestive-related organs	0.001	0.001
12	"	Pancreas	0.001	0.001
25	"	Prostate	0.025	0.039
33	"	Lung small cell carcinoma	0.043	0.015
	White female			
1	"	All sites	0.048	0.002
2	"	All digestive	0.038	0.001
3	"	Digestive tract	—	0.002
4	"	Esophagus	0.007	0.004
5	"	Stomach	0.001	0.001
8	"	Rectum	—	0.044
9	"	Digestive-related organs	0.004	0.004
12	"	Pancreas	0.001	0.002
13	"	Retroperitoneum	0.036	0.014
15	"	All respiratory	0.001	0.001
17	"	Trachea, bronchus, lung	0.001	0.001
18	"	Pleura	0.027	0.035
20	"	Breast	0.005	0.002

nately, this does not allow identifying the exact location of the association of ingested asbestos and cancer in the body.

These findings in no way lend themselves to the interpretation regarding the possible regulation of asbestos in drinking water. Only research of the direct method design would allow for such conclusions. The recommendations from this research is that more investigation of a direct nature be done regarding digestive cancers and ingested asbestos. The scientific community will then be better equipped to answer questions about the possible regulation of asbestos in drinking water.

The author expresses appreciation to Steve Selvin and Deane W. Merrill for providing the census population density data. The advice and support of James Millette are gratefully acknowledged.

This work was supported by U.S. Environmental Protection Agency Grant No. C2455NTET.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Kanarek, M. S., Conforti, P. M., Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer incidence in the San Francisco Bay Area. Am. J. Epidemiol. 112:54–72 (1980).
2. Conforti, P. M., Kanarek, M. S., Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer in the San Francisco Bay Area: 1969–1974 incidence. J. Chron. Dis. 34:211–224 (1981).
3. McCarthy, J. L., Merrill, D. W., Marcus, A., Benson, W. H., Gey, F. C., Holmes, H., and Quong, C. The socio-economic-environmental demographic information system. Computer Science and Mathematics Department, Lawrence Berkeley Laboratory, University of California, Berkeley, 1981.
4. U.S. Department of Commerce, Bureau of the Census: 1970 Census of Population and Housing, Census Tracts San Francisco–Oakland, California SMSA. PHC(1)-189. U.S. Government Printing Office, Washington, DC, 1972.

Environmental Health Perspectives
Vol. 53, pp. 79–89, 1983

A Graphical Analysis of the Interrelationships among Waterborne Asbestos, Digestive System Cancer and Population Density

by Michael E. Tarter,* Robert C. Cooper,*† and William R. Freeman‡

Five statistical procedures were used to partial the correlation between waterborne asbestos and digestive site cancer for the putative effects of population density. These include: analysis based on a data subset with roughly homogeneous population density; standard residual analysis (partial correlation); conditional probability integral transformation; analysis based upon ranked data, and use of logarithmic transformation.

Nonparametric regression graphical techniques are applied to examine the nature or shape of the asbestos-cancer dose-response curve. Evidence is presented that suggests that there is considerable difference between analyses involving nonhigh-density tracts and non-San Francisco tracts. Evidence is also presented that the modal-type nonparametric regression curve forks or bifurcates when adjustment is made for population density.

Introduction

In 1979, a study was completed by Cooper et al. (*1*), which dealt with certain health effects of asbestos in the domestic water supplies of five California counties. Several publications (*2,3*) and even a lawsuit involving the State of Connecticut and asbestos pipe producers have resulted in part from the tentative findings of the Cooper study that "a statistical association between asbestos fiber content in drinking water and the incidence of certain cancers in the San Francisco Bay Area has been shown."

In testimony presented during the Connecticut hearings, one witness suggested that the results of the Cooper-Kanarek et al. study were suspect due to lack of adjustment for, or partialling of, the population density variate. This paper describes a follow-up investigation that was undertaken to deal with the questions involving the population density putative intervening variate.

The material presented here is not reported upon in order of scientific significance, but instead the simplest methodological approach is described first and subsequent results are organized in terms of the complexity of the statistical methodology used. For example, an investigation in which the original Cooper et al. (*1*) study is repeated on a subset of low and moderately populated census tracts is described. By far the most interesting and dramatic reported here it is that presented at the end of this paper, which relies on a complex type of nonparametric regression (one based on a locus of conditional density modes). It demonstrates that the morbidity density may be bimodal for high asbestos exposure and unimodal for low asbestos exposure census tracts.

Exclusion of the Highly Dense Tracts

The estimated distribution of census tract subgrouping population density variate Y is highly

*Department of Biomedical and Environmental Health Sciences, University of California at Berkeley, Berkeley, CA 94720

†Sanitary Engineering and Environmental Health Research Laboratory, University of California at Berkeley, Berkeley, CA 94720.

‡Graphical Biometry Laboratory, University of California at Berkeley, Berkeley, CA 94720.

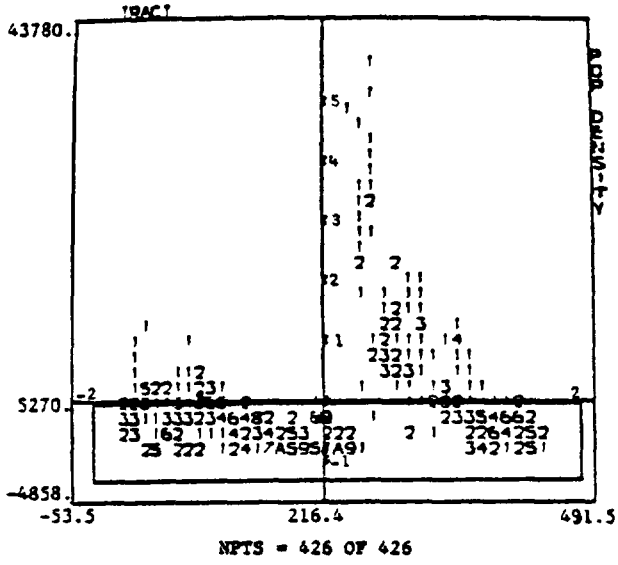


FIGURE 1. Population density-census tract frequency diagram.

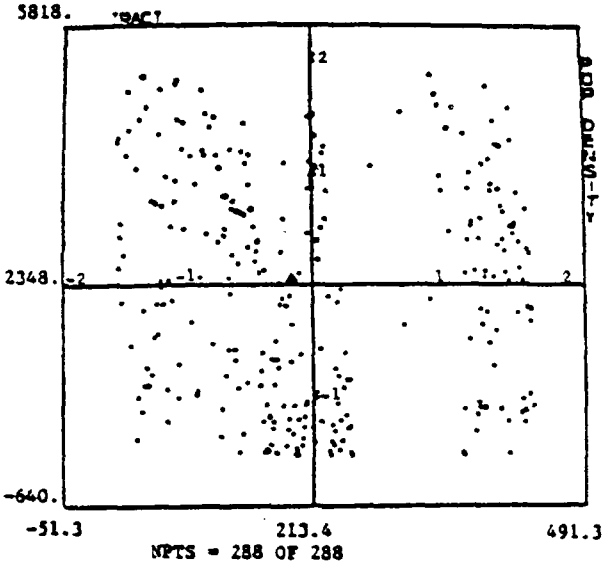


FIGURE 2. Low to moderate density scatter diagram.

skewed. Figure 1 displays the frequency distribution of Y and the independent variate tract number code X. To avoid confusion (e.g., between 11 coincident points and 2 nearby single points, each represented by the symbol '1'), a frequency of 10 is represented by the character A, 11 by B, 12 by C, and so on up to 35 (Z) and more (*). As an indication of skewness, note that while most census tracts contain less than the mean $E(Y) \equiv 5270$ individuals per square mile, several census tracts are at least six times more densely populated than the mean. Relative to tracts where Y takes on a value > 5270, tracts with $Y < 5270$ form an almost homogeneous subgrouping. Furthermore, tract number (the X variate of Fig. 1) is a rough measure of geographic location. Hence, the Bay Area population seems to consist of the following three components: (1) a highly dense component lying slightly to the right of the mean tract number $X = E(X) \equiv 216$ line; (2) a moderately dense component below component 1 but above the $Y = E(Y) \equiv 5270$ line; and finally (3) a large group of tracts with moderate to low densities, shown in Figure 1 enclosed by an editing rectangle.

When the region enclosed by the editing rectangle is enlarged and a scatter diagram of the region is displayed as in Figure 2, there appears to be a moderately homogeneous spread of Tract POP DENSITY points in most portions of the display not immediately to the right of the $X = 216$ line.

Studies of the variate log population density (described below) suggest that there may be two distinct classifications within what appears by eye to be one homogeneous grouping of low to moderate density census tracts. Nevertheless, as a preliminary analysis, it seems reasonable to repeat the basic Cooper et al. (*1*) study with the use of the low to moderate population density tracts.

There were 288 tracts with low to moderate population density. Figure 3 is a frequency di-

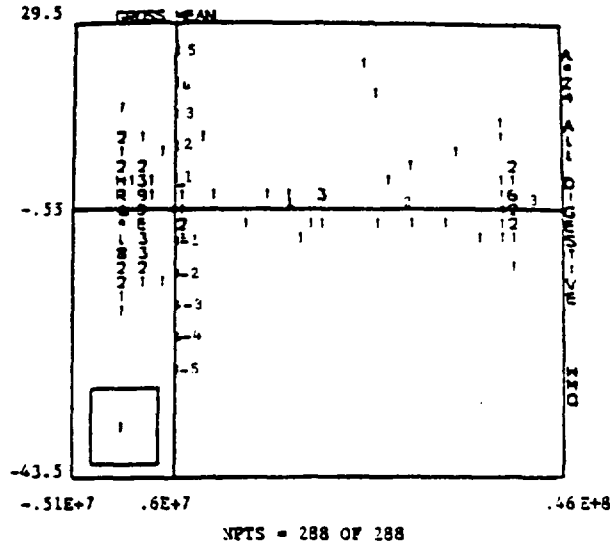


FIGURE 3. Low to moderate density asbestos-excess cancer frequency diagram.

gram obtained by plotting the variate asbestos exposure in fibers/liter (indicated as "gross mean" in the figure) on the x-axis and the variate observed minus expected (i.e., excess) white male all digestive site per tract cancer on the y-axis (*1*). One census tract whose coordinates are shown enclosed in an editing rectangle appears to have either an extremely low observed cancer morbidity or an extremely high expected cancer morbidity. The position of the point that represents this tract implies that, were this tract to be removed, the Pearsonian correlation between the X and Y variates would be reduced. Nevertheless, despite the removal of this point, and much more importantly the removal of all high-density tracts from consideration, the correlation between asbestos exposure and excess cancer morbidity variates is 0.194. Even for a one-sided test, the α level for the null hypothesis of no association would have to be smaller than 0.005 for one to accept the null hypothesis in this case. Thus one can be reasonably sure that the exclusion of highly populated tracts from the original study of Cooper et al. (*1*) would not change the study's principal finding of a significant relationship between waterborne asbestos exposure and digestive tract cancer. It might be noted that the correlation between asbestos exposure and excess morbidity for the entire data set of low, medium, and high-density tracts is 0.241.

Regression Studies

In a previous study (*3*), nonparametric regression procedures were applied in order to study the shape of the dose-response curve associated with the asbestos exposure-cancer excess morbidity relationship. In this section it will be demonstrated that the previously observed linearity of the dose-response curve seems to persist for low to moderate exposure levels and low to moderately populated tracts.

Following the approach used in Tarter (*3*) as a preliminary to the analysis of the 287 low to moderate density census tracts described by Figure 4, the margins between the leftmost and rightmost data points and the endpoints of the interval of estimation were reduced. This operation was performed for the purpose of taking advantage of the periodic nature of Fourier series nonparametric representation. The effects of this margin reduction are shown in Figure 5, which can be thought of as an enlargement of Figure 4.

The conventional least-squares fitted regression line obtained from the 287 low to moderate density points is shown in Figure 6, as are the 95% confidence bands associated with this line. These bands enclose a region within which, provided certain basic assumptions are satisfied (*4*), one is 95% sure that the true dose-response curve is contained. The fact that no horizontal, i.e., null relationship, line is contained within the confidence bands is the graphical equivalent of the dose-response significant relationship described in the previous section.

Unlike the parametric regression line shown in Figure 6, nonparametric regression procedures do not rely on the initial assumption that the dose-response curve conforms to any particular functional relationship e.g., line. However, the flexi-

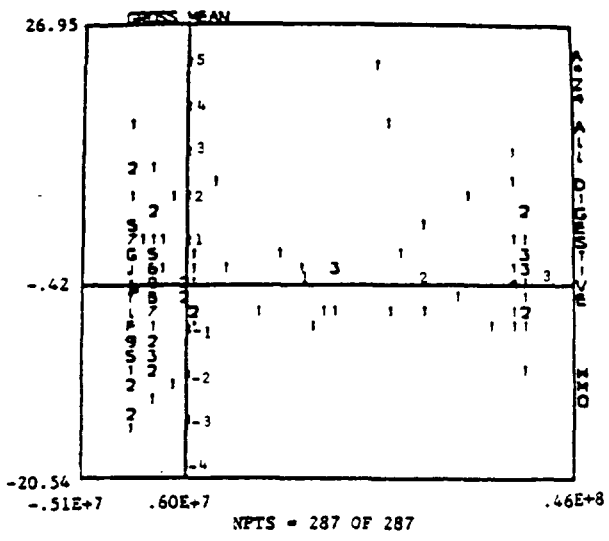


FIGURE 4. Frequency diagram after removal of outliers.

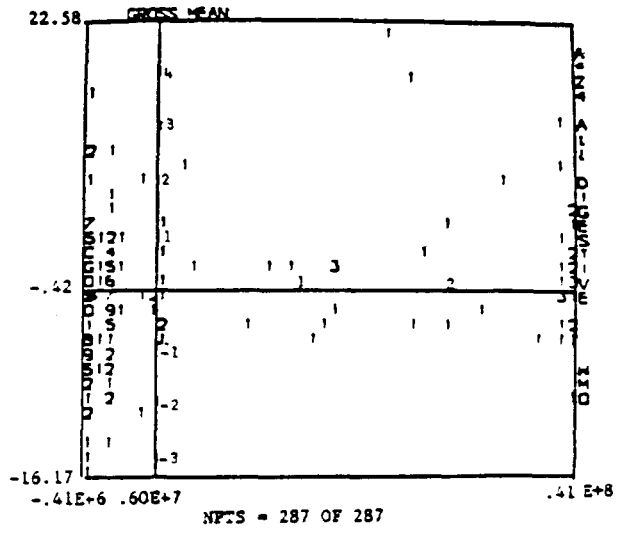


FIGURE 5. Margin-reduced frequency diagram.

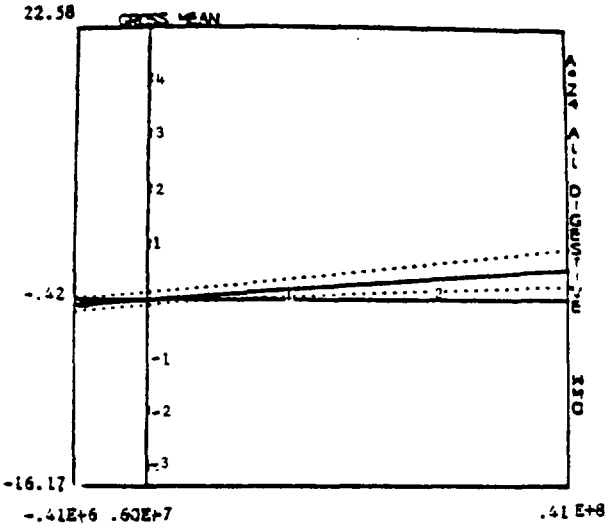


FIGURE 6. Regression line with confidence bands.

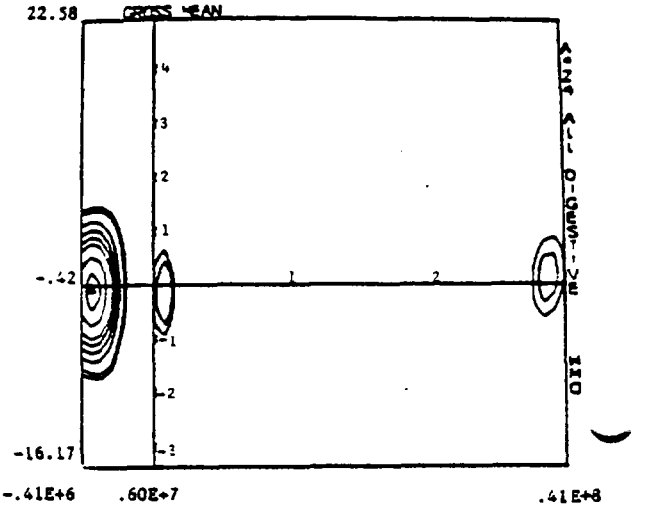


FIGURE 7. Contour diagram showing sparse-rich data distribution.

bility inherent in nonparametric methodology makes it extremely difficult to obtain useful estimates within subregions where data points are sparsely distributed. Approaches that can be used to augment conventional procedures and effectively deal with sparse-rich data mixtures are described by Breiman et al. (*5*), Tarter (*6*), and Bean and Tsokos (*7*).

Figure 7 is a contour diagram that illustrates the uneven distribution of asbestos exposure-excess cancer morbidity data. To obtain Figure 7 a generalized three-dimensional histogram was constructed above the gross mean asbestos exposure and excess digestive cancer sample space. This estimated frequency surface was then sectioned parallel to the sample space to produce the contours, i.e., terraces, isopleths, shown in Figure 7. The three outermost contours correspond to loci of points whose estimated relative frequency is exactly 30% of the modal frequency, i.e., the most common single combination of exposure and excess morbidity.

Naturally, the nonparametric regression curve shown in Figure 8, which was obtained from the same estimated bivariate frequency used to obtain Figure 7, exhibits a highly irregular pattern. The procedure used to obtain this curve has been described in Tarter (*8*).

To increase the resolving power of the nonparametric estimation procedure, a technique was employed that increases the second moment of all density components in the x-direction but that leaves all other distributional characteristics unchanged. This procedure is a variant of a spectral

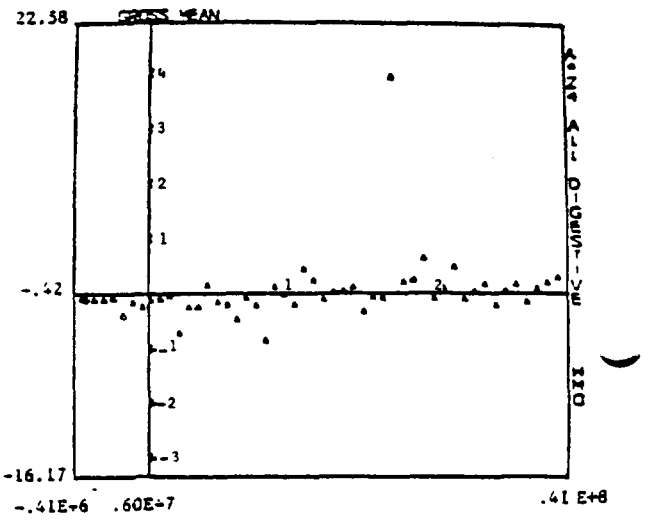


FIGURE 8. Nonparametric regression before smoothing.

analysis technique described by Medgyessy (*9*) and generalized to two dimensions by Stanat (*10*) and Tarter and Silvers (*11*). Figure 9 is the analog of Figure 7 produced by smoothing in the x-direction, while Figure 10 is the analog to Figure 8.

As depicted by the indicator or pointer line, the nonparametric regression curve seems to reach a plateau or horizontal component at coordinates X = 27,500,000 fibers/L and Y = 0.51 excess cases over a 6-yr period per census tract. In other words, when only the low to medium density tracts are considered, there is no statistical evidence that a change of exposures to any value in

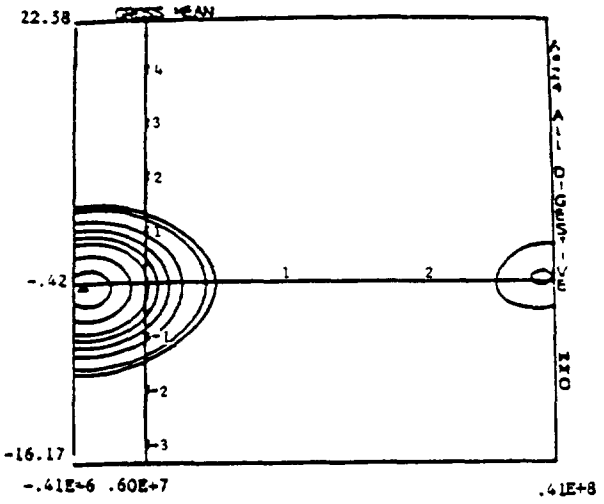


FIGURE 9. Equiprobability contours after smoothing.

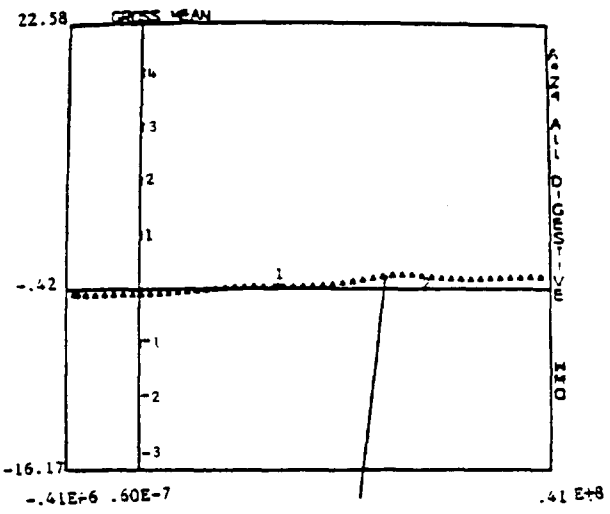


FIGURE 10. Nonparametric regression after smoothing.

excess of 27,500,000 fibers/L will increase the estimated dose-response curve to more than 0.51 excess cases. This finding is of course far from surprising, since there are so few high-exposure tracts among the 287 tracts with low to medium population density.

By using procedures described by Tarter (*3*), benchmark data confirmation procedures were applied to check whether the statistical methods used to obtain Figure 8 could have influenced the ramp as opposed to step i.e, diagonal as opposed to horizontal shape of the nonparametric regression curve. These procedures tend to confirm that not only is the relationship between waterborne asbestos level and excess cancer statistically significant for the low to moderate population density subsample, but there exists concomitant variation (*12*) between these two variates up to the point $X = 27{,}500{,}000$, $Y = 0.51$. When nonparametric regression procedures other than that used to obtain Figure 8 were applied to these data (*13*), the same slight but discernible concomitant increase in Y with an increase in X less than 27,500,000 fibers/L was observed.

Residual and Partialling Studies

The most straightforward and elementary procedure usually used to remove the effects of a single underlying or nuisance variable is of course residual linear regression or its near equivalent, partial linear correlation. One can view this procedure in terms of a process that involves the following two basic steps. (1) One or both variables to be partialled are used as response or dependent Y variates, and the variate Z whose effect is to be removed is fitted (usually by least-squares methods) to Y. Most commonly a regression slope $\hat{\beta}$ is obtained such that Y and $\hat{\beta}Z$ have maximum correlation. Up to a constant, usually denoted by a, $\hat{\beta}Z$ "explains" as much of the variation of Y as is possible by any linear combination $a + \hat{\beta}Z$. (2) The residual $Y - \hat{\beta}Z$ is used to replace the response variate Y, and in some instances the same procedure involving a second value of $\hat{\beta}$ is used to find the residual of principal independent variable X and nuisance independent variable Z. For the problem at hand, Y represents excess cancer morbidity, X asbestos exposure level, and Z population density. The variate $Y - \hat{\beta}Z$ is sometimes interpreted as that part of Y that has not been "explained" by the variate Z (*14*).

For the 426 original census tracts of Cooper et al. (*1*), Figure 11 shows the result of applying the above residual regression technique. To better interpret this figure, a code has been used in our GRAFSTAT program (*15–18*), which on the right-hand vertical column specifies B*Z2 A*Z1 ALL DIGESTIVE WMO. To interpret this graph legend, note that the basic Y variate was the ALL DIGESTIVE site white male observed (hence WMO) per census tract number of cases per 6-yr period. The prefix A*Z1 designates that the A (linear or additive) transformation has been applied to the Z1 variate ALL DIGESTIVE WME, where the last "E" designates that it was the expected number of cancer cases that was substituted for the observed cases to form, after subtraction from its WMO counterpart, the basic Y variate, excess morbidity.

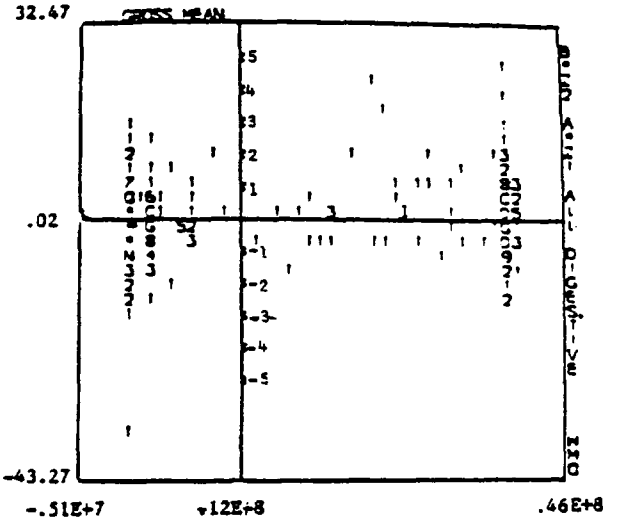


FIGURE 11. Standard partialled frequency diagram.

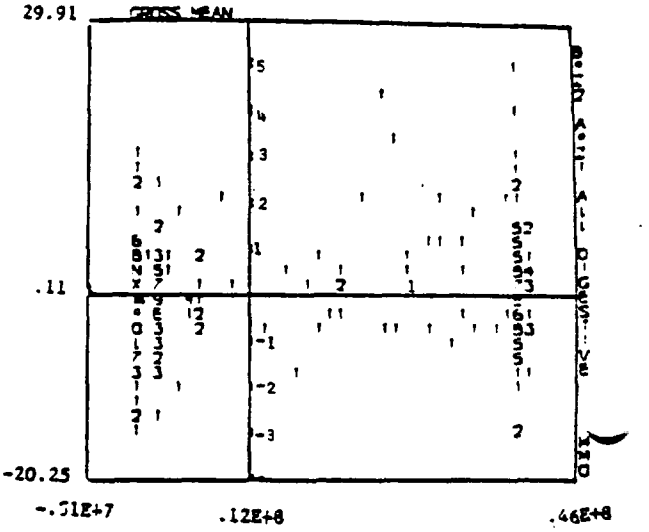


FIGURE 12. Partialled frequency diagram after removal of outliers.

The outer prefix B*Z2 designates that after the variate excess morbidity had been formed the least-squares residual (transformation B) was used to obtain that part of the A*Z1 ALL DIGESTIVE variate that was not linearly explained by the Z2 variate, population density.

A comparison of Figures 11 and 3 shows a very close correspondence between the B transformed complete data set and the untransformed low to medium density data subset at all but the rightmost column of data points. The same lower left-hand corner putative outlier is present in these two figures, and the correlation between the two variates is $r = 0.171$, which for a sample of size $n = 426$ is significant at the $\alpha = 0.01$ level.

It should be mentioned that we have repeated all the experiments described in this paper with the X variate gross mean asbestos exposure adjusted for population density by the same technique used to adjust the Y variate, morbidity. For ease of interpretation, experiments involving a partialled or residual Y variate and an unpartialled (origial) X variate will be presented here.

Data with the single lower leftmost putative outlier removed from the file were used to obtain Figure 12 and an associated correlation of $r = 0.171$.

Conditional Probability Integral Partialling

The partialling or residual approach described in the preceding section has the advantages of being well studied and easily interpreted provided certain initial specifications or assumptions are satisfied. The so-called "problem of specification" (*19*) underlies a considerable literature usually denoted by the terms nonparametric, model-free, or curve estimation. Hence, it seems very reasonable to consider nonparametric alternatives to simple linear-residual analysis.

As will be described later in detail, the variate population density has a highly nonnormal and skewed distribution. Hence, the first of the wide variety of model-free approaches applied involved a technique known as a conditional probabili integral transformation (*20*, *21*) whose effectiveness is minimally affected by distributional specification.

Unfortunately the price paid for generality is a certain difficulty in interpretation. The variate I*Z2 A*Z1 ALL DIGESTIVE WMO, where the letter I designates that the conditional probability integral information (CPIT), has been applied to remove the effect of population density, has a correlation of $r = 0.133$ with the asbestos exposure variate, as shown in Figure 13. It is interesting that the positive correlation persists despite the drastic nature of the CPIT transform. However, it is too early in the study of the CPIT method to accurately assess the statistical significance of the correlation in this case.

Parametric-Nonparametric Hybrids

Two approaches were used to take into account the skewed nature of the density variate and yet

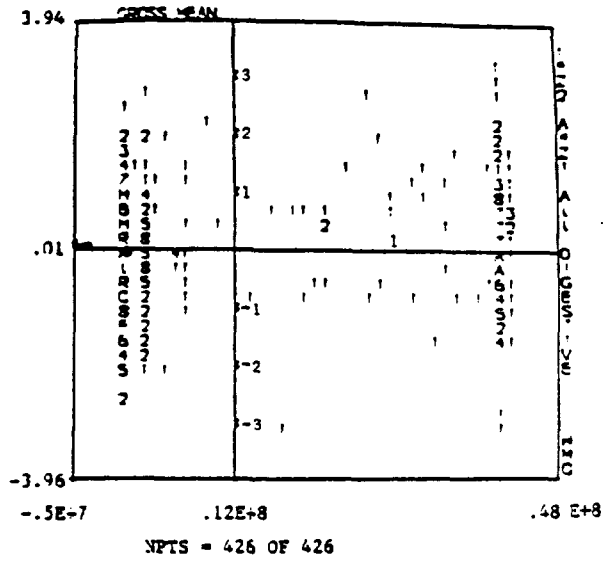


FIGURE 13. Conditional probability integral partialled frequency diagram.

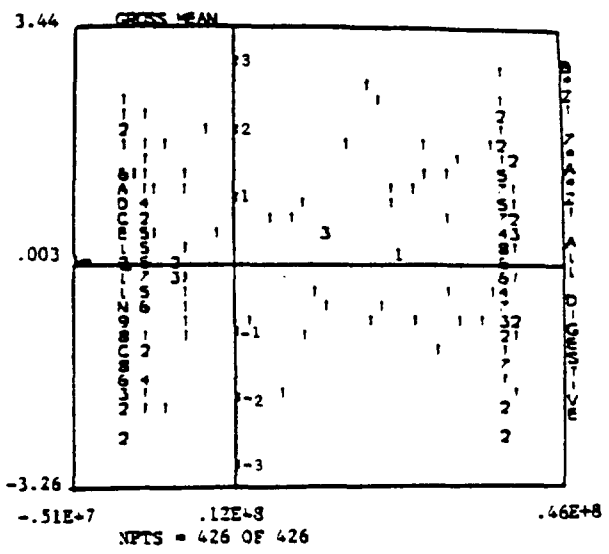


FIGURE 14. Partialled and ranked data.

obtain a result more easily interpreted in terms of statistical significance than the result described in the previous section. The first involved a commonly used nonparametric approach based on the rank transformation (*22*), and the second the logarithmic transformation (*3*).

The number 7 shown in the Y axis label of Figure 14 designates the seventh GRAFSTAT data transformation option, normalized rank or score (*4*). For a fixed value of nuisance variable Z, e.g., population density, this option assures that

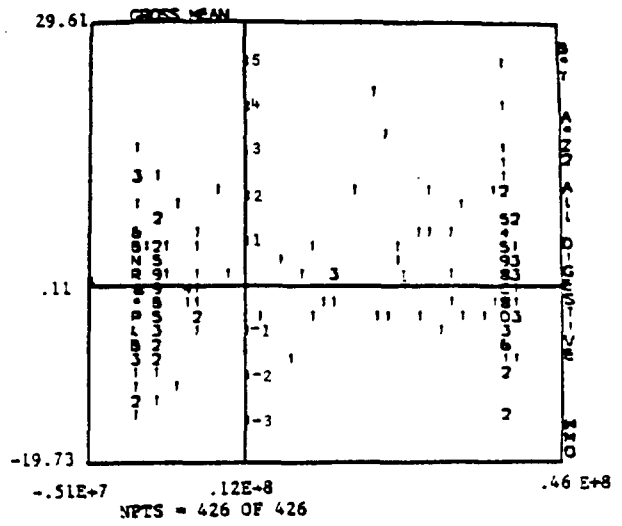


FIGURE 15. Partialled and log transformed data.

the order between any two transformed values matches that of any two untransformed values, and that the distribution of the transformed score is approximately standard Gaussian which, among other things, guarantees that the variate's distribution is no longer skewed. The correlation in this case is $r = 0.143$.

It should be pointed out that although the distribution of both the 7*A*Z1 ALL DIGESTIVE WMO variate and the 7*POP DENSITY variate can now be treated as if they were Gaussian, the joint distribution of Y and X need not be bivariate normal. Nevertheless, as described by Tarter and Kowalski (*21*), the use of the normalized score transform does seem to be useful in a wide variety of contexts with the proviso, as suggested by the Figure 14 Y variate prefix B*Z1, that the simple least-squares difference between the normalized variates is sufficient to remove all density effect.

To obtain Figure 15, the variate log POP DENSITY was used in place of the ranked variate 7*POP DENSITY. The similarity between Figure 11 and Figure 10 and that between the corresponding correlations of $r = 0.157$ and $r = 0.143$ suggests that in this case the correlation is highly robust with respect to changes in the X and Y variate individually. The difference between the above correlation and the correlation associated with the CPIT I transformation suggests that the relationship between the X and Y variates may be highly complex. One possible cause of this complexity could be the existence of disjoint distributional subcomponents for either the morbidity or population density variate.

Univariate Investigation of the Population Density Variate

Figure 16 is a histogram constructed from the 426 census tracts. The variate whose density is estimated by this histogram is the logarithm of the per-tract population density. Figure 17 is a nonparametric kernel-Fourier series estimator (23), which suggests that the gap between the leftmost and second-leftmost mode of the histogram in Figure 16 is not an artifact traceable to sampling variation. This brings into question the low to moderate population density homogeneity that underlies the analysis.

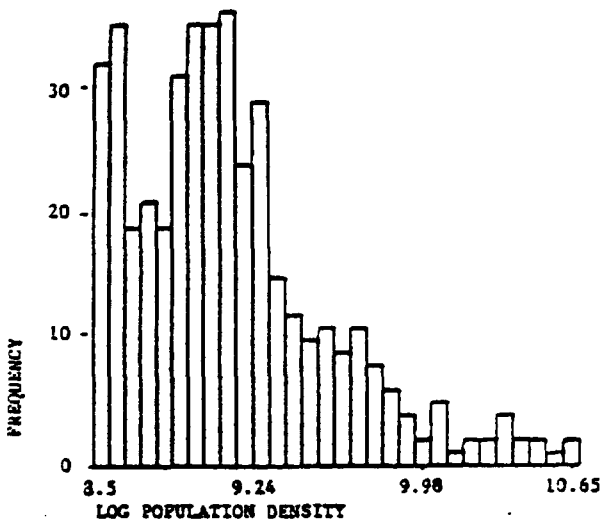


FIGURE 16. Log population density histogram.

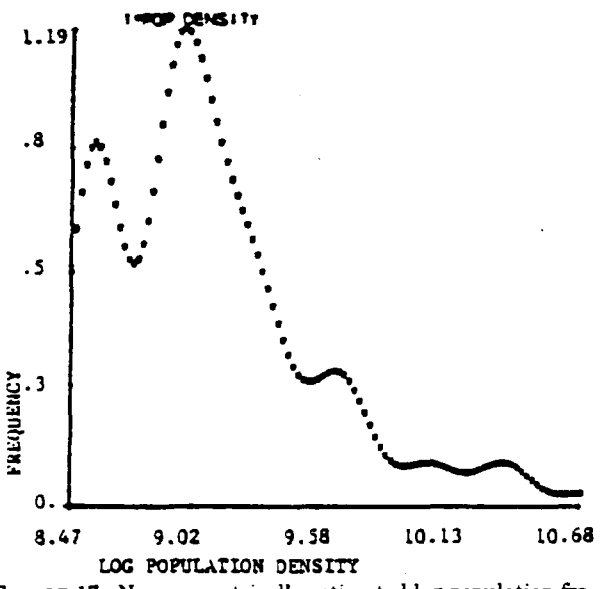


FIGURE 17. Nonparametrically estimated log population frequency curve.

Study of Non-San Francisco Tracts

Because of the complexity of the population density variate, it was decided to repeat the investigation described above after excluding tracts within the city limits of San Francisco. The relationship between the basic asbestos exposure X variate and the observed per (non-San Francisco) tract white male all digestive organ morbidity is described by Figure 18. The correlation between these two variates is $r = 0.136$. (Here sample si $n = 323$, i.e., there were 323 non-San Francisco tracts.)

Once the Z1 variate, expected morbidity, was subtracted from the observed morbidity, Figure 19 was obtained. Now, however, the correlation between X and Y has dropped to the nonstatistically significant value of $r = 0.05$. Thus, one can be reasonably sure that the significant relationship between asbestos and cancer is largely attributable to those low to moderately populated tracts located in San Francisco. Tentative findings have already been published involving several San Francisco tracts (24) which suggests that there may be data collection or spurious correlation artifacts associated with a particular San Francisco subregion.

To carefully study the non-San Francisco tracts, the lower left outlier was again removed as show

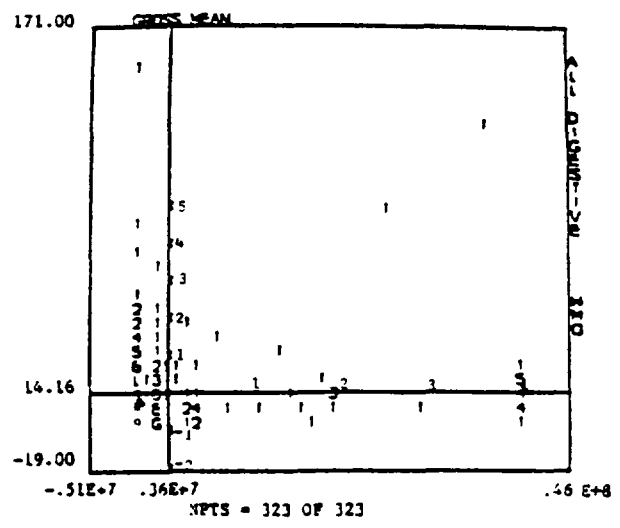


FIGURE 18. Non-San Francisco observed cancer-asbestos exposure frequency diagram.

cated in San Francisco, it seems that the relationship between waterborne asbestos exposure and excess digestive cancer morbidity may be traceable to a greater proportion of tracts associated with code 1 as shown in Figure 25 than tracts associated with code 2, i.e., the bimodal density of morbidity has a higher mode associated with high than with low morbidity as shown in Figure 25, where the number 1 is used to represent the higher and number 2 the lower mode. Hypothetically, suppose that there exist two types of census tracts, type 1 (higher mode) and type 2 (lower mode), the former with a greater and the latter with a lesser probability of an individual contracting digestive cancer. It appears from Figure 25 that the high asbestos exposure subgroup seems to have a greater preponderance of type 1 individuals than does the low to moderate exposure subpopulation. Of course, as suggested in the previous two sections, this finding may not have anything to do with a causal connection between asbestos and cancer. It may simply be a coincidence that type 1 tracts tend to be clustered in that part of the Bay Area that happens to have high exposure levels.

Despite the above caution, it should be pointed out that the rather striking nature of Figure 25 was only uncovered after a partialling technique was applied to remove the effect of population density. Thus, rather than being an artifact traceable to the underlying effects of population density, the relationship between asbestos levels and excess cancer morbidity was brought more clearly into focus once population density was taken into account.

The authors would like to thank Seemin Qayum and Joe Rodrique for their technical assistance. This research was supported by grant 1 RO1 CA 28142-01A2 from the National Cancer Institute, National Institutes of Health.

Preparation of this manuscript was supported by contract 68033130 from the U.S. Environmental Protection Agency.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Cooper, R. C., Tarter, M. E., Kanarek, M. S., Murchio, J. K., Conforti, P. M., Jackson, L. A., Callard, R., and Lysmer, D. Asbestos in Domestic Water Supplies in Five California Counties. EHS Publication No. 79-1, Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, 1979.
2. Kanarek, M. S., Conforti, P. M., Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer incidence in the Bay Area. Am. J. Epidemiol. 112: 54–72 (1980).
3. Tarter, M. E. Pattern recognition in the context of an asbestos cancer threshold study. In: Proceedings of the Thirteenth Annual Symposium on the Interface: Computer Science and Statistics (W. F. Eddy, Ed.), Springer-Verlag, New York, 1981, pp. 105–110.
4. Dixon, W. J., and Massey, F. J. Introduction to Statistical Analysis. McGraw-Hill, New York, 3rd ed., 1969.
5. Breiman, L., Meisel, W., and Purcell, E. Variable kernel estimates of multivariate densities. Technometrics 19: 135–144 (1977).
6. Tarter, M. E. Biocomputational methodology: an adjunct to theory and applications. Biometrics 35: 9–24 (1979).
7. Bean, S., and Tsokos, C. P. Bandwidth selection procedures for kernel density estimates. Commun. Statist. Theory Methods 11: 1045–1069 (1982).
8. Tarter, M. E. Trigonometric maximum likelihood estimation and application to the analysis of incomplete survival information. J. Am. Statist. Assoc. 74: 132–139 (1979).
9. Medgyessy, P. Decomposition of Superpositions of Distribution Functions. Hungarian Academy of Sciences Publishing House, Budapest, 1961.
10. Stanat, D. F. Unsupervised learning of mixtures of probability functions. In: Pattern Recognition (L. N. Kanal, Ed.), Thompson, Washington, DC, 1968, pp. 357–389.
11. Tarter, M. E., and Silvers, A. Implementation and applications of bivariate Gaussian mixture decomposition. J. Am. Statist. Assoc. 70: 47–55 (1975).
12. Mill, J. S. A System of Logic, Ratiocinative and Inductive. Longmans, Green, Reader, and Dyer, London, 8th ed., 1872, pp. 464–466.
13. Fisz, M. Probability Theory and Mathematical Statistics. John Wiley, New York, 3rd ed., 1963, pp. 91–96.
14. Croxton, F. E., and Cowden, D. Applied General Statistics. Prentice-Hall, New York, 1940, p. 743.
15. Tarter, M. E., Rigsbee, E. O., and Wong, J. T. Interactive editing of biomedical data. Computer Programs Biomed. 6: 117–123 (1976).
16. Tarter, M. E., Marshall, J. S., Lum, S. B., Rigsbee, E. O., and Wong, J. T. A description of the Berkeley Graphical Biometry Project. In: Proceedings of the Ninth Interface Symposium on Computer Science and Statistics (D. C. Hoaglin and R. E. Welsch, Eds.), Prindle, Weber and Schmidt, Boston, 1976, pp. 44–51.
17. Tarter, M. E. A description of the GRAFSTAT clinical life table options. In: Proceedings of the Statistical Computing Section, The American Statistical Association, Washington, DC, 1978, pp. 44–58.
18. Tarter, M. E. Modular implementation of graphical curve estimation procedures. In: Proceedings of the Statistical Computing Section, The American Statistical Association, Washington, DC, 1981, pp. 69–78.
19. Tapia, R. A., and Thompson, J. R. Nonparametric Probability Density Estimation. Johns Hopkins University Press, Baltimore, 1978, pp. 21–22.
20. Quesenberry, C. P., and Starbuck, R. R. On optimal tests for separate hypotheses and conditional probability integral transformations. Commun. Statist. Theory Methods A5: 507–524 (1976).
21. Tarter, M. E., and Kowalski, C. J. A new test for and class of transformations to normality. Technometrics 14: 735–744 (1972).
22. Kendall, M. G. Rank Correlation Methods. Hafner, New York, 3rd ed., 1962.
23. Tarter, M. E., and Kronmal, R. A. An introduction to the implementation and theory of nonparametric density estimation. Am. Statistician 30: 105–112 (1976).
24. Tarter, M. E. Comment on Good and Gaskins. J. Am. Statist. Assoc. 75: 63–66 (1980).

Environmental Health Perspectives
Vol. 53, pp. 91–98, 1983

Epidemiology Study of the Use of Asbestos-Cement Pipe for the Distribution of Drinking Water in Escambia County, Florida

by James R. Millette,* Gunther F. Craun,* Judy A. Stober,* Dale F. Kraemer,*† H. G. Tousignant,‡** Ellen Hildago,‡ Robert L. Duboise‡ and Jeffrey Benedict*

Cancer mortality for the population census tracts of Escambia County, FL, which use asbestos-cement (AC) pipe for public potable water distribution, was compared with cancer mortality data collected from census tracts in the same county where other types of piping materials are used. An analysis of covariance was run to test for differences in standard mortality ratios for seven cancer sites among three potential asbestos exposure groups based on AC pipe usage. Twelve variables representing nonexposure-related influences on disease rates were combined in four independent factors and used as covariates in these analyses. No evidence for an association between the use of AC pipe for carrying drinking water and deaths due to gastrointestinal and related cancers was found. The limitations on the sensitivity of the analysis are discussed.

Introduction

Concern over the presence of asbestos fibers in drinking water supplies began in 1973 after millions of mineral fibers per liter were reported in the drinking water in Duluth, MN (*1, 2*). The question of possible increased risk of disease resulting from long-term ingestion of asbestos fibers in drinking waters is important because the association between some occupationally exposed asbestos workers and increased risk of respiratory and digestive disease has been documented (*3, 4*).

In June 1974, electron microscopy confirmed that fibrous material recovered from a kitchen faucet strainer in Pensacola, FL, was asbestos. While the source of mineral fibers in the Duluth water supply was related to industrial discharge of mining wastes, the fibers in the Pensacola water appeared to be connected with the deterioration of asbestos-cement (AC) distribution mains. Because AC pipe is used to carry drinking water throughout the United States, it was decided that a study of people should be conducted, by census tracts, in an area where there was evidence of fibers coming from the pipe.

Study Area

Escambia County is located on the Gulf Coast in the upper panhandle of Florida. A little over 200,000 people live in the county, which is divided demographically into 40 census tracts. Some of the tracts have been receiving water through AC pipe for 30–40 yr, other tracts have been serviced by nothing but cast iron pipe, and still others receive water primarily from private wells with no AC pipe. Data on water quality from 25 wells showed unfinished waters in the county to be very similar, all with the chemical characteristics that make water corrosive to most types of common plumbing materials as well as to unprotected water mains. Comparison of water quality data from 1951 and 1957 and 1975 data showed that the well source waters had changed little in their

*Toxicology and Microbiology Division, USEPA, 26 West St. Clair Street, Cincinnati, OH 45268.
†Present address: University of Iowa, Iowa City, IA 52242.
‡Escambia County Health Department, 2251 North Palafox Street, Pensacola, FL 32574.
**Retired.

chemical characteristics over the years. With the assistance of Escambia County Health Department personnel, an exposure assessment and a compilation of cancer mortality data were conducted in simultaneous but separate projects to determine whether or not the people in the county using AC pipes showed a greater risk of disease.

Exposure Evaluation

Seven water samples were taken over the period of a year from the Montclair water distribution system, where the asbestos material had been found in a kitchen faucet strainer. The drinking water was analyzed by newly developed electron microscopic techniques (*5*). As seen in Table 1, varying amounts of asbestos fibers were found in the consumers' drinking water. The absence of fibers in the well samples indicated that the asbestos fibers found in the distribution system probably originated in the AC pipe. The first sampling point, Residence 1, was in a house on the older part of the water system, and Residence 2, in a house on the newest part. During the sampling period, fiber concentrations in the water may have been influenced by changing treatment practices at the well source; utility personnel monitored the system more closely and increased the dosage of lime in an attempt to make the water less corrosive to AC pipe. Later inspections of excavated sections of pipe confirmed that the pipe was releasing fibers, as it showed rather severe deterioration of the interior pipe surface. Some of the chemical qualities of the water that caused it to be so aggressive were low pH (5.2), low calcium hardness (1.4 mg/L as $CaCO_3$), and low alkalinity (1 mg/L as $CaCO_3$). According to current American Water Works Association standards, these qualities indicate water that, if left untreated, would be highly corrosive and might eventually destroy the structural integrity of AC pipe. In earlier days, however, AC pipe was selected because it was thought to be free from the corrosion problems that were experienced with metal pipe, and its use in corrosive water situations had eliminated red water (iron corrosion) complaints.

Discussions with local utility personnel indicated that the pipe deterioration of the Montclair system was not truly representative of all the AC pipe in Escambia County. The Montclair system, established in 1957 and expanded through 1968, was privately owned originally and apparently its water was not treated consistently. There were numerous consumer complaints about waterborne fibers, clogged customer meters, and problems at a coin-operated laundry, all of which were attributed to fiber release from the AC pipe. An inspection of the Montclair treatment facilities on June 4, 1964, by the county health department disclosed no stabilization treatment equipment in operation and a "heavy solid load in the water in the form of asbestos fibers." When the system was acquired by the municipality in 1968, lime treatment to reduce corrosiveness was started on a continuous basis, and the system was flushed and interconnected with the overall city system. Most customer complaints subsided after the municipal acquisition but, occasionally, visible clumps of fibers were recovered, as in the June 1974 case.

Utility records for the greater part of Escambia County did not show AC pipe unearthed from areas other than the Montclair system to be as severely deteriorated as the pipe in that system. The average raw water quality in 1976 for the 25 wells in the Pensacola City system showed a pH

Table 1. Asbestos fiber counts for the Montclair System sample points.

Date of sample	Fiber counts, 10^6 fibers/L[a,b]					
	Well		Residence 1		Residence 2	
	Amphibole	Chrysotile	Amphibole	Chrysotile	Amphibole	Chrysotile
01/17/75	BDL (0.01)	0.02	0.01	1.7	0.2	1.2
02/21/75	BDL (0.01)	NSS (0.06)	NSS (0.03)	32.7	BDL (0.02)	0.4
03/26/75	BDL (0.01)	NSS (0.01)	NSS (0.1)	1.7	BDL (0.02)	0.3
05/09/75	BDL (0.01)	NSS (0.01)	BDL (0.01)	1.2	BDL (0.02)	0.7
07/07/75	BDL (0.01)	NSS (0.06)	NSS (0.06)	0.7	BDL (0.01)	0.1
09/05/75	BDL (0.01)	NSS (0.01)	NSS (0.06)	0.2	BDL (0.01)	BDL (0.01)
12/04/75	BDL (0.01)	BDL (0.01)	BDL (0.01)	0.4	BDL (0.01)	BDL (0.01)
12/08/77	BDL (0.05)	NSS (0.05)	BDL (0.05)	NSS (0.01)	NSS (0.1)	NSS (0.2)
01/29/79	c	c	01	0.7	c	c

[a]BDL = Below detectable limit (shown in parentheses).
[b]NSS = Not statistically significant. Value shown in parentheses is based on too few fibers found in analysis to be considered accurate.
[c]Not sampled.

of 5.2, a calcium hardness of 18 mg/L as $CaCO_3$, and an alkalinity of 2.5 mg/L as $CaCO_3$. While this suggests a highly corrosive water, each well was equipped with a lime treatment system designed, not necessarily to stabilize the water, but to make the distributed water less corrosive. Monitoring the multitude of mechanical feeders was not, however, an easy task, and the corrosiveness of the water varied over the years. A sample taken from the distribution system of Pensacola in 1951 for the Florida State Board of Health showed that the treated water had a tendency to be corrosive, with a pH of 7.3, a calcium hardness of 25 mg/L as $CaCO_3$ and an alkalinity of 30 mg/L as $CaCO_3$. The Langlier index of this sample was determined to be -1.6. The water itself was clear with no sediment. Prior to 1977, the occurrence of corrosive water was also evidenced by red water complaints from areas that used cast iron pipe. In 1977, pH recorders with hi-lo alarms were installed at the well plants and were connected to a central control room. Red water complaints decreased drastically after this careful monitoring began.

The Escambia County Health Department was asked to collect water samples from residences that they felt to be representative of the water systems in the county. The analysis results of all the samples collected are shown in Table 2. Some water samples taken from census tracts using AC pipe showed measurable asbestos concentrations, while others did not. The increase in the lime dosages in many of the individual well plants after 1975 may have had an influence on fiber counts. The changing water flow patterns throughout the connected systems may also have caused waters from a system with one type of pipe to flow into another. Care was taken to sample only from consumers' homes rather than from fire hydrants, and samples were not taken when work on the pipe was underway near the sampling site. Chemical analyses of water samples collected in December 1977 (Table 3) showed that many of the waters at the consumers' taps still had the capability to be moderately corrosive. In a system where a corrosive water is attacking AC piping material, the water would change chemically and become less corrosive as it moved through the system. Although there was some indication of pH and calcium change in the water as it flowed through the Montclair system, water in other systems in the county could not be studied closely for this effect because of changing treatment practices at each well. There is some evidence from other studies that iron entering the water from the corrosion of cast iron lines near the source of a

Table 2. Asbestos fiber counts from homes in various census tracts.

Census tract	Sample number	Date of sample	Concn, 10^6 fibers/L[a,b] Amphibole		Chrysotile		Principal area pipe characteristics
3	39719	12/07/77	BDL	(0.05)	BDL	(0.05)	Fe
4	39720	12/07/77	BDL	(0.05)	BDL	(0.05)	Fe
6	39716	12/07/77	BDL	(0.03)	NSS	(0.16)	Fe
9	39717	12/07/77	BDL	(0.05)	NSS	(0.24)	Fe
10	39718	12/07/77	BDL	(0.05)	BDL	(0.05)	50% AC/50% Fe
13	39725	12/08/77	NSS	(0.24)	NSS	(0.24)	AC
22	39730	12/08/77	NSS	(0.16)	NSS	(0.16)	AC
23	39729	12/08/77	BDL	(0.02)	NSS	(0.12)	AC
27	35454	08/10/76	NSS	(0.1)		0.4	AC
29	39721	12/07/77	BDL	(0.05)	NSS	(0.24)	AC
30	39722	12/07/77	BDL	(0.05)	NSS	(0.24)	AC
31	39723	12/08/77	BDL	(0.05)	NSS	(0.16)	AC
32	39724	12/08/77	NSS	(0.24)	NSS	(0.24)	AC
33	40615	04/14/76	NSS	(0.1)		3.2	AC
33	35457	08/10/76		0.1		10.7	AC
33	39728	12/08/77	BDL	(0.02)	NSS	(0.12)	AC
33	39754	01/29/79	NSS	(0.05)		0.7	AC
34	40617	04/14/76	NSS	(0.1)		0.7	AC
35	40616	04/14/76	BDL	(0.01)	NSS	(0.05)	AC
35	35455	08/10/76	BDL	(0.02)		0.1	AC
35	35455	08/10/76		0.5		4.7	AC
35	39726	12/08/77	BDL	(0.03)		0.16	AC
37	39727	12/08/77	BDL	(0.03)	NSS	(0.16)	Fe

[a]BDL = Below detectable limit (shown in parentheses).
[b]NSS = Not statistically significant. Value shown in parentheses is based on too few fibers found in analysis to be considered accurate.

Table 3. Some water quality values from home faucet samples collected 12/7–8/77.

Census tract	Sample number	Field pH	Lab pH	Alkalinity, mg/L	Calcium, mg/L	Total iron, mg/L	AI[a]	Principal area pipe characteristics
3	39719	6.6	7.2	31	37.5	0.45	10.3	Fe
3	39725	7.8	7.7	27	29.7		10.6	AC
4	39720	6.8	7.5	32	25.5	0.46	10.4	Fe
6	39716	7.0	7.6	44	59	0.17	10.7	Fe
9	39717	6.2	6.6	9	13	0.07	8.7	Fe
10	39718	5.7	6.6	9	8.3	0.07	8.5	50% AC/50% Fe
22	39730	6.8	7.5	30	30.5		10.5	AC
23	39729	6.8	7.6	36	35.0		10.7	AC
29	39721	8.0	8.1	34	41.3	0.07	11.2	AC
30	39722	7.6	7.7	36	43.7	0.07	10.8	AC
31	39723	8.4	8.4	31	34.5		10.4	AC
32	39724	7.8	7.9	34	38.3		11.0	AC
33	39728	6.4	7.1	15	15		9.4	AC
35	39726	7.4	7.5	26	28.2		10.4	AC
37	39727	9.6	9.0	50	53.7		12.4	AC

[a]AI = Aggressive Index = pH + log (AH), where A = total alkalinity in mg/L as $CaCO_3$ and H = calcium hardness in mg/L as $CaCO_2$.

system may have a protective effect on AC pipe further on in the distribution system. There were no utility reports of iron coatings on AC pipe unearthed. Nevertheless, the reports of red water problems suggest this may have occurred in some areas.

Data from analysis of the water samples showed asbestos fibers in the drinking waters of some areas. However, the changing water treatment practices at the well plants over the years prevented these data and the data from later samplings to be used specifically to estimate past exposures. Instead, the Escambia County census tracts were divided into three potential exposure groups based on years of use and amount of AC pipe, as follows.

Potential High Exposure. At least 90% of the population was served by AC pipe for 25 yr or more in 1976 (9 tracts). Tract numbers: 13, 17, 19, 20, 27, 29, 30, 31, 33; population: 46, 123.

Low Exposure. Less than 90% of the tract population was served by AC pipe or, if over 90% of the population was served by AC pipe, the pipe was less than 25 yr old in 1976 (13 tracts). Tract numbers: 3, 10, 11, 12, 14, 18, 21, 26, 28, 32, 34, 35, 36; population: 86,897.

No Exposure. No AC pipe was used in the census tract. Either cast iron pipe was used, or the tract population was not on public water supply (14 tracts). Tract numbers: 1, 2, 4, 5, 6, 7, 8, 9, 15, 16, 37, 38, 39, 40; population: 51,378.

Figures 1 and 2 show maps of the geographic distribution of the census tracts assigned to different potential exposure classification groups. It should be noted that census tracts 1, 2, 4, 5, 6 and 7 are in the city of Pensacola and census tracts 38, 39 and 40 are in a rural area of the county. Census tract 37 is centered around a papermill.

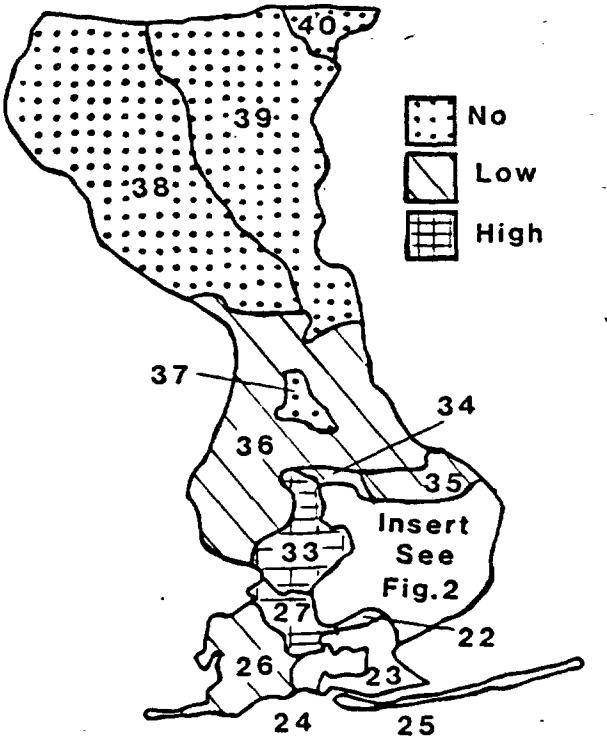


Figure 1. Census tracts in Escambia County, FL, with an indication of exposure to asbestos-cement pipe used for the delivery of drinking water.

Utility personnel indicated that all AC pipe in the county was thought to be type II pipe. Pipe of this classification is autoclaved and is more corro-

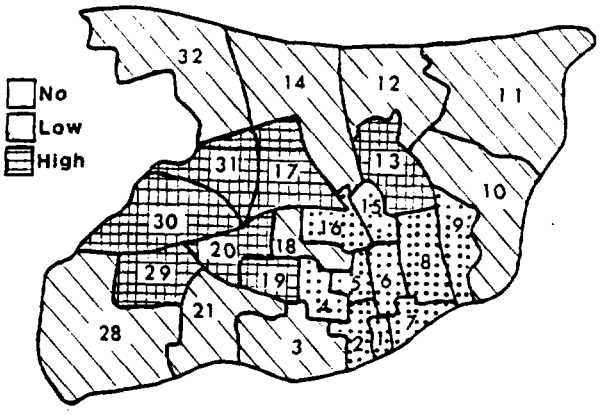


FIGURE 2. Detail of Figure 1. Central county census tracts with an indication of the exposure to asbestos-cement pipe.

sion resistant than type I pipe. Only populations exposed for 25 yr or more were included in the potentially high exposure category because studies of occupationally exposed asbestos workers show a latency period of at least 20 yr between initial exposure and onset of disease (*6*). Because most of the systems are interconnected, water may have, on occasion, flowed from a system with one type of piping into a system with a different type of piping. Although this factor could not be considered when assigning census tracts to potential exposure groups, it probably did not significantly alter exposure patterns over any great length of time. Health Department personnel determined that the flow would usually be from systems with iron pipe to those with AC pipe when mixing did occur. The Montclair system is found in both census tracts 31 and 32. The newer AC pipe section is in tract 32. Four census tracts (total population: 20,936) were not included. Tract 25 is a beach area where housing is mostly less than 5 yr old. Tract 24 is a naval base with their own water supply. Tracts 22 and 23 were not included because Escambia County Health Department officials felt that the tracts would have very high migration patterns because the residents are mostly Navy personnel.

Cancer Data

Because neither a tumor registry existed for Escambia County nor were morbidity statistics available, an Escambia County Health Department official examined all death certificates from 1963 to 1976 by hand. Cancer deaths for the sites listed in Table 4 were recorded by year for each census tract along with data from the death certificate including age, sex and race (white or nonwhite).

A sex-site specific standard mortality ratio (SMR), i.e., the ratio of the expected to observed deaths, was calculated for each tract by using the 1970 U.S. population age-sex-race-site specific rates as standard rates. Eight age categories (0–24, 25–34, 35–44, 45–54, 55–59, 60–64, 65–74, 75 and older) and two race categories (white and nonwhite) were used.

Other Variables

Data on other variables that might influence cancer disease rates were obtained for each census tract. Table 5 lists 12 variables that provided some measure for the parameters of socioeconomic status, occupation, residence stability, and population density. The socioeconomic variables included 1970 census data (*7*) from mean income (INCOME); percent of households below the poverty level (PHOUSE); percentage of high school graduates (PHSG); median year of school com-

Table 4. Summary of raw data for actual numbers of deaths (1963–1976) by exposure group for all age groups, races, both sexes.

		Exposure group		
	Code[a]	No	Low	High
1970 Population	—	51378	86897	46123
Bladder	188	32	26	23
Kidneys	189	5	10	8
Gastrointestinal[b]	150–159	155	128	131
Pancreas	157	66	61	58
Liver	155	16	17	15
Lungs	162	256	253	277
Other, excluding skin	170–171, 174, 190–199	448	371	350

[a]International Classification of Disease for Oncology, WHO, 1976.
[b]Includes esophagus, stomach, intestines, colon, rectum, liver, gallbladder, pancreas, pertitoneum.

Table 5. Socioeconomic indicators.

Code	Description
1. INCOME	Mean income of census tract
2. PHOUSE	% households below poverty
3. PLABOR	% in total labor force
4. PCONST	% working in construction
5. PMANUF	% working in manufacturing
6. PHSG	% high school graduate
7. SHOUSE	% resident same house in 1970 as in 1965
8. OHOUSE	% resident in other house same SMSA
9. PTRANP	% working in transportation
10. SEX	Male/female ratio in census tract
11. PMYR	Median school year complete
12. POPDEN	Population density

pleted (PMYR); and percentage of the work force working in construction (PCONST), manufacturing (PMANUF), or transportation (PTRANP). According to Escambia County Health Department information, the county had no asbestos product manufacturing companies. Data on the number of residents in the same house in 1965 as in 1970 (SHOUSE) and in the same area (Standard Metropolitan Statistical Area) (OHOUSE) were chosen to represent population stability. Population density (POPD), which has been cited as a potentially important factor in other studies of ingested asbestos, was calculated from data supplied by the West Florida Regional Planning Council.

Data Analysis

For each of the cancer types listed in Table 4, an analysis of covariance (*8,9*) was run to test for differences in SMRs among potential exposure groups (high, low, no). In order to adequately satisfy the assumptions needed for an analyses of covariance (e.g., normality), the square root of the SMR was used as the dependent variable. The 12 highly interrelated nonexposure variables listed and defined in Table 5 were reduced to four uncorrelated composite variables by principal components analysis. These new variables (factor scores), representing a composite of nonexposure-related influences on disease rates, were used as covariates in these analyses (*8,10*). Factor 1 was basically a socioeconomic class variable. The main contributions to this factor were from the variables INCOME, PHOUSE, PHSG, and PMYR. Factor 2 was made up of mainly SEX and PLABOR (which was the only variable other than sex to differ between the sexes) and is the sex class variable. Factor 3 was made up of mainly SHOUSE, OHOUSE, POPDEN, PCONST, and reflected some measure of mobility. Factor 4 was made up of mainly PMANUF and PTRANP and reflected these job classes.

Results

As seen in Table 6, there were no cancer sites for which the SMRs differed significantly ($p < 0.05$ level) between census tract groups having potentially different asbestos fiber concentrations in their waters. The R^2 values for all sites were less than 0.25 except for the site category of "other."

Discussion

The results of the analyses in this study do not show any statistical association between the deaths due to certain cancer types and the use of AC pipe in Escambia County, FL. This conclusion is consistent with the results of a previous study of cancer incidence in Connecticut towns using AC pipe (*11*). No other published epidemiologic studies have specifically considered AC pipe.

In this type of epidemiology study, it is not possible to assess the possible influences of such individual factors as smoking, occupation, and alcohol consumption on disease. These factors have been discussed in previously published studies of the same type that considered exposure to asbestos in drinking water (*12,13*). The R^2 values, which indicated that less than 25% of the variation between exposure groups was accounted for by the general census tract level factors considered, were similar to the R^2 values of the other studies (*11–13*). Only the composite factor 4 showed a regression coefficient significantly different from zero for any sites ($p < 0.05$). This factor is most heavily influenced by the job classes of manufacturing and transportation, but the in-

Table 6. Exposure group mean SMR values for cancer sites.

Potential exposure	SMR							
	GI	Pancreas	Kidneys	Bladder	Urinary[a]	Liver	Lungs	Other[b]
Adjusted mean SMR (square root transformation)								
High	0.721	0.848	0.221	0.632	0.551	0.925	1.092	1.671
Low	0.651	1.013	0.296	0.839	0.722	1.04	1.087	1.676
No	0.596	0.822	0.339	0.638	0.602	0.727	0.903	1.555
Probability value	0.184	0.462	0.758	0.511	0.477	0.7389	0.245	0.546
R^2	0.21	0.17	0.15	0.11	0.14	0.01	0.16	0.46
Mean SMR values								
High	0.563	0.989	0.330	0.850	0.590	2.132	1.256	2.753
Low	0.456	1.083	0.417	1.026	0.687	1.987	1.178	2.698
No	0.407	1.074	0.173	0.744	0.554	1.329	1.035	2.947

[a]Combined kidneys and bladder.
[b]Other unspecified sites.

terpretation of its statistical significance is difficult because the other nonexposure variables also have some effect on the markup of this factor.

The size of the population involved in this study is an important factor. For those cancers with low frequencies of occurrence, the results must be interpreted carefully, since one or two deaths could change the results enough to alter the conclusions. In this study, mortality due to kidney cancer did not occur in great numbers. Of the census tracts, 75% had no deaths from kidney cancer over the 14-yr period studied. Four census tracts had recorded two deaths from kidney cancer; two were in the high exposure group and one each in the low and no exposure groups.

It is apparent from the maps in Figures 1 and 2 that the census tracts assigned to the various exposure groups are also grouped geographically. This is an obvious consequence of studying piping materials in a connected water distribution system. Whether this physical alignment of tracts is a confounding bias could not be readily assessed with existing data. Attempts were made, however, to control for socioeconomic differences that might be related both to the geographic groupings and disease mortality. In previous studies of asbestos in water, the population density and population mobility were found to be important factors (*12, 13*). As seen in Table 7, the "no" exposure group, containing both the central city census tracts of Pensacola and the rural tracts in the northern part of the county, had both the highest and lowest census tract population densities. The range of densities within this exposure group was over three orders of magnitude. The other two exposure groups had a much smaller range of population density values. Analyzing the data without considering population density had some effect on the adjusted SMRs but did not alter the results. The direct effect of population density itself was not measured since independent composite factors were used as covariates. Differences among SMRs in the analyses of the gastrointestinal cancer site were greater when population density was included in the analysis.

Population mobility may be especially important in asbestos-related cancer studies due to the long latency period between initial exposure and onset of disease. As indicated in one study (*12*), population movement into, out of, and within an area could conceivably result in findings that either showed an association where none existed or masked a real difference in cancer associated with the use of AC pipe. As can be seen in Table 7, population stability, as measured by the percentage of population 5 yr old or older living in the same house in 1970 as in 1965, was similar for the three exposure groups. All three were near the 47% level of the "high stability" super tracts analyzed in the California Bay Area asbestos study (*12*). The fact that about 50% of the people had moved in a 5-yr period casts doubts on the usefulness of this type of study for diseases with long latency periods. Acknowledging that some people may move several times in a period of years, the data in Table 7 suggest that possibly less than one-tenth of the people in the "possible high" exposure census tract group in the 1970s were actually using water delivered by AC pipe 25 yr earlier.

The sensitivity of the statistical analyses is an important consideration. The study should have been able to detect a 70% increase in gastrointestinal cancer mortality in the population with high exposure to AC pipe versus the population with no exposure ($\alpha = 0.05$). However, the ability to identify an association, if one existed, with the individual cancer sites of kidneys, bladder or liver was poor. Because of the small numbers of deaths due to cancer of those sites, a 300% increase would not have been identified as statistically significant.

It is useful to compare the Escambia County situation with the calculations on ingested asbestos risk made from occupational data and described in a proposed ambient water quality criteria document (*14*). This exercise suggests that ingestion of 300,000 asbestos fibers/L will result in one cancer death among 100,000 people in 70 years. The validity of calculating this risk value from occupational groups exposed to airborne asbestos is open to question, and the use of it in this

Table 7. Population density and stability.

	Population density[a]				Stability, %[b]		
Exposure group	Median	Mean	Max.	Min.	Mean	Max.	Min.
No	130	3,950	33,000	10	60%	72%	52%
Low	795	1,800	5,500	70	44%	64%	33%
Possible high	1,033	3,200	4,000	300	48%	59%	36%

[a]Density: 1970 census tract population/square miles of census tract.
[b]Population in the same house, 1965/population 5 yr or older in 1970.

calculation should in no way imply its acceptance as a standard-setting value. It is, however, a useful value for evaluating epidemiologic studies of ingested asbestos. The criterion is equivalent to predicting 0.5 cancer cases/million people per year per million fibers per liter. If it is assumed that the exposure level was between 1 and 10 million fibers/L (Table 2) for the exposed population of about 50,000 people, there will be estimated an additional 0.03 to 0.3 cancer cases per year. This would be an additional four deaths maximum to be added to the 14-yr data in Table 4. This increase would be on the order of 2% for gastrointestinal cancer deaths. If the additional cancers estimated by the criterion had occurred, they would not have been detected in the present study. The above calculation requires a number of assumptions, including that of a lifetime (70-yr) exposure for the population at risk. A paper in these proceedings (*15*) has pointed out that the actual average exposure of a population at a given point in time is 31 years. Consideration of this fact serves to decrease the estimated number of cancer deaths that would be predicted and therefore decreases further the chance that an effect could have been detected in this study if in fact an effect was present.

Conclusion

To the level of sensitivity imposed by the limitations described in the discussion, this study found no evidence for an association between the use of AC pipe for carrying drinking water and deaths due to gastrointestinal and related cancers in Escambia County, FL.

The authors thank Mr. Gib Newsome from the Escambia County Health Department; Mr. Buddy Page from the West Florida Regional Planning Council; Mr. G. Michael, Ms. C. Sonich, Ms. M. Snyder, and Mr. P. Clark from the USEPA in Cincinnati; and Mr. Shiferaw Gebremariam formerly with the University of Cincinnati, Department of Biostatistics, for their help on this study.

The complete census tract population breakdown by race, sex, and age necessary for analysis was contained in the Complete Second Count Data of the 1970 Census of Population and Housing for Escambia County, FL, obtained by contract from the National Planning Data Corporation, Ithaca, NY.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Nicholson, W. J. Analysis of amphibole asbestiform fibers in municipal water supplies. Environ. Health Perspect. 9: 165–172 (1974).
2. Cook, P. M., Glass, G. E., and Tucker, J. H. Asbestiform amphibole mineral: detection and mesurement of high concentrations in municipal water supplies. Science 185: 853–855 (1974).
3. Selikoff, I. J., and Hammond, E. C. Multiple risk factors in environmental cancer. In: Persons at High Risk of Canc (J. F. Fraumeni, Ed.), Academic Press, New York, 197 pp. 467–483.
4. Enterline, P. E., and Kendrick, M. A. Asbestos-dust exposure at various levels and mortality. Arch. Environ. Health 15: 181–186 (1967).
5. McFarren, E. F., Millette, J. R., and Lishka, R. J. Asbestos analysis by electron microscope. Proc. Am. Water Works Assoc. Water Quality Technology Conf. 14: 1–12 (1975).
6. Selikoff, I. J., Hammond, E. C., and Seidman, H. Latency of asbestos disease among insulation workers in the United States and Canada. Cancer 46: 2736–2740 (1980).
7. 1970 National Census, Pensacola, FL, SMSA.
8. Kleinbaum, D. G., and Kupper, L. L. Applied regression analysis and other multivariable methods. Duxbury Press, North Scituate, MA, 1978.
9. Jennrich, R., and Sampson, R. Analysis of variance and covariance including repeated measures. In: BMDP Biomedical Computer Programs, P-Series (W. J. Dixon and J. B. Brown, Eds.), BMDP-79, University of California Press, 1979, pp. 540–548.
10. Sall, J. P. "Factor" in SAS Users Guide, 1979 Edition (Helwig and Council, Eds.), SAS Institute, Cary, NC 1979, pp. 209–210.
11. Meigs, J. W., Walter, S. D., Heston, J. F., Millette, J. R., Craun, G. F., Woodhull, R. S., and Flannery, J. T. Asbestos cement pipe and cancer in Connecticut 1955–1974. J. Environ. Health. 42: 187–191 (1980).
12. Conforti, P. M., Kanarek, M. S., Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer in the San Francisco Bay area 1969–1974 incidence. J. Chron. Dis. 34: 211–224 (1981).
13. Polissar, L., Severson, R. K., Boatman, E. S., and Thomas, D. B. Cancer incidence in relation to asbestos in drinking water in the Puget Sound region. Am. J. Epidemiol. 116: 314–328 (1982).
14. U.S. Environmental Protection Agency. Water Quality Criteria: Asbestos. Fed. Reg. 44: 56632–56635 (1979).
15. Erdreich, L. Comparing epidemiology studies of ingested asbestos for risk assessment. Environ. Health Perspect. 53: 99–104 (1983).

Environmental Health Perspectives
Vol. 53, pp. 99–104, 1983

Comparing Epidemiologic Studies of Ingested Asbestos for Use in Risk Assessment

by Linda S. Erdreich*

Epidemiologic data can be used in risk assessment in various ways: to calculate the dose-response relationship between exposure levels and adverse effects; to establish ranges of exposure known to be safe or unsafe; to verify an endpoint in humans derived from a route or species extrapolation; to support assumptions necessary for performing extrapolation procedures. These points are illustrated in the risk assessment for exposure to asbestos in drinking water.

A previous risk assessment for asbestos, the EPA's Ambient Water Quality Criteria (AWQC) for Asbestos, was derived from cohort studies of inhalation exposure. Epidemiologic studies of ingested asbestos, all of geographical correlation design, were compared on the basis of their ability to add information in support of both the route extrapolation and low-dose extrapolation used in this risk assessment. Results of these ingestion studies were inconsistent due to variable ability to detect a risk from chronic low-level exposure. After preliminary comparisons of factors that determine scientific validity and statistical power, two ingestion studies were selected to determine if they had the potential to detect the risk predicted by the AWQC.

This evaluation has shown that these studies do not offer quantitative data for estimating levels associated with a defined risk. Due to short exposure duration and limited power, clearly safe and clearly unsafe ranges could not be definitely identified. The most appropriate ingestion studies suggest, but do not prove, the endpoint derived from the route extrapolation in the AWQC.

Introduction

The incorporation of information from epidemiologic studies into health risk assessment depends on the nature of the available scientific data and the nature of the unresolved issues. Epidemiologic data can be used in health risk assessment for a variety of purposes. The process of screening and comparing epidemiologic studies for use in health risk assessment will be described for ingested asbestos.

The EPA's Ambient Water Criterion (AWQC) for Asbestos for human health is based on human inhalation studies; therefore, data on the health effects of human exposure via ingestion are necessary to verify this route extrapolation. Ingested asbestos studies are evaluated to select those appropriate for use in risk assessment. Central to this evaluation is the comparison of the increased risk predicted on the basis of the AWQC with the estimated ability of a given study to detect this risk.

An integral aspect of screening studies for use in risk estimation is the critical analysis of all the strengths and weaknesses of relevant aspects of each study. Because Marsh, in his presentation at this workshop (*1*), did this in great detail for the epidemiologic studies of ingested asbestos, this analysis will not be repeated here.

Background

One of EPA's many endeavors in the area of health risk assessment has been the Ambient Water Quality Criteria Documents (AWQCD) (*2*). The Clean Water Act of 1977 (P.L. 95-217) required these documents to calculate criteria for 65 toxic pollutants or pollutant categories and to summarize and reflect accurately current knowledge of the kind and extent of all effects on human health that would be expected from the presence of pollutants in ambient water. The water quality criteria for human health are a scientific assess-

*Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency, 26 West St. Clair, Cincinnati, OH 45268.

ment of ambient levels of a pollutant that is estimated to result in defined levels of risk for a given carcinogen or no-observed-adverse-effect levels for toxicants. States may use these criteria and, after considering local environmental conditions and human exposure patterns, incorporate them into regulations as standards.

Obviously, the ideal source of data for assessing human risk is exposure situations in humans via the appropriate exposure route. In the absence of quantitative route-specific data for humans, it is sometimes necessary to substitute either human data from another route of exposure or dose-response data in animals. Extrapolations from route to route (e.g., inhalation to ingestion), from animals to humans, or from high to low doses are performed according to procedures documented in the *Federal Register* (*3*). These procedures are constantly being refined and apply to situations in addition to those of ambient water quality—hazardous waste sites, for example.

Basis of the Current AWQC for Asbestos

The AWQC for asbestos is derived from exposure-response data in three epidemiologic studies involving large occupational cohorts exposed for a working lifetime to airborne asbestos. These workers, who were followed over time to ascertain causes of death, were found to have an increased incidence of peritoneal mesothelioma and gastrointestinal (GI) cancers. In two of the studies used to calculate the criterion, GI cancers included esophagus, stomach, small intestine, colon and rectum. In the third study, liver, gallbladder and pancreas were also included. The assumption that these cancers can also be caused by ingested asbestos was supported by positive associations for digestive tract sites in the geographic correlation study of ingested asbestos in the California study first reported by Kanarek et al. (*4*). The route extrapolation is also supported by the fact that most of the inhaled asbestos is cleared from the respiratory tract and ingested by normal body processes.

The average of the excess deaths in all three cohorts exposed via inhalation was related to the average exposure index. This average was also extrapolated to low dose levels to estimate criteria levels; e.g., the ambient water level necessary to keep lifetime excess cancer risk to an exposed population below 10^{-5} is 0.3 million fibers of all sizes/L (0.3 MFL), assuming ingestion of 2 L water/day and 70 yr exposure (*2*). According to EPA guidelines (*3*), the extrapolation to low dose is linear when using human data and does not include calculation of an upper 95% confidence limit as when the extrapolation is calculated from animal data. Because both a route extrapolation and a low-dose extrapolation were used, the criteria would be strengthened by support from human studies of exposure by ingestion at ambient levels.

Uses of Epidemiologic Data in Risk Assessment

Epidemiologic data can be used in risk assessment for a variety of purposes: (1) to estimate t dose-response relationship in order to identify the highest concentration level at which no adverse health effects or a defined low-level of adverse health effects are anticipated; (2) to establish ranges of exposures that are or are not associated with an adverse effect; (3) to support or verify an endpoint identified from a different route of exposure or from experiments in animals; (4) to support general assumptions necessary for performing extrapolation procedures.

These purposes depend on characteristics of the data as determined by study design, power, and quality of the exposure data. For example, quantitative exposure-response data are necessary for quantitative estimates described in points (1) and (2). Studies with qualitative exposure data are useful for point (3) to verify a route extrapolation or a target organ for a suspected carcinogen. To support extrapolation processes, point (4) usually requires a body of evidence, such as the human epidemiologic studies of carcinogens that correlate with animal studies.

The derivation of the existing asbestos AWQC for human health from epidemiologic data illustrates point (*1*). That assessment required an extrapolation from the inhalation exposure route to the ingestion route because existing epidemiologic studies of ingested asbestos were of geographic correlation (ecological) design not applicable for quantitative risk assessment.

Research on the health effects of asbestos has continued since the publication of the AWQC and has contributed information that can be used to reevaluate this risk assessment. More recent studies designed to evaluate the association between ingested asbestos and cancer can be used to address point (*2*), distinguishing safe from unsafe ranges of exposure, and point (*3*), verifying the association between ingested asbestos and certain cancers. Point (*4*) describes the use of a broad epidemiologic data base and is presented for com-

pleteness, although data on asbestos cannot yet be used for this purpose.

Comparing Epidemiological Studies of Ingested Asbestos

All nine ingestion studies compared in this discussion are ecological studies in which readily accessible vital statistics, such as mortality or incidence, are correlated with the exposures assumed to be experienced by these populations. Geographic correlation studies usually serve as screening devices to indicate associations that could be examined subsequently with more precise and powerful analytic designs, such as case control or cohort. The correlation studies were performed to offer a rapid and cost-efficient mechanism for addressing the public health questions of whether areas with asbestos-cement pipe have higher cancer rates. The nine studies cited (*4–12*), which were performed in five geographic areas, lack precision due to the possible misclassifications inherent to this study design in which residence in an area is assumed to reflect lifetime exposure to that water supply. Inferences that can be made are weak and insufficient for establishing a causal relationship. These studies do, however, make it feasible to incorporate a sample size sufficiently large to detect small increases in low-probability outcomes such as cancer.

The limitations of these asbestos studies are due in part to characteristics inherent to the problem of studying the effects of low-level exposures on rare outcomes such as cancer. Even at the high occupational exposure levels, relative risks for mortality following inhalation exposures ranged from 1.5 to 2.5 for GI sites (*13*). The limitations listed in Table 1 suggest that, to establish the dose-response gradient by epidemiologic studies, an analytic (case-control or cohort) study of sufficient sample size would be required to detect the low relative risk expected.

This table also lists approaches to handle these limitations. Many have been attempted in the studies to date. For example, a surveillance system has been initiated in Duluth to follow trends over time (*5*), and studies were initiated in the Puget Sound area, which has high ambient levels (*11*). Confounding risk factors for cancer, such as diet, smoking habits and occupational exposures, are not well evaluated in geographic correlation studies.

The evidence for a causal relationship between a risk factor and disease is often evaluated by comparing the pattern of results among studies. Consistent and repeatable results to support the association are particularly important when the association is weak due to low exposure levels or the low potency of the carcinogen. Table 2 shows that few associations between asbestos and cancer are positive in both sexes, and few are repeated across studies for the same cancer site (*11*). Two sites that show repeated positive associations among studies are the pancreas and stomach, but the latter is not consistently positive for both sexes. Since the AWQC was based on total peritoneal mesotheliomas and "gastrointestinal cancers," mainly of esophagus, stomach, colon and rectum, those sites must be examined together in order to evaluate the AWQC. (Peritoneal mesothelioma is rare and was often omitted from the studies because few cases were reported.)

Table 3 presents results for these sites, as reported only in papers representing two of the areas, Duluth and California (*4–6,9*). Mortality data for Duluth reported for four 5-yr periods did not support an increase, although the data were tested for trend (*5*). Levy et al. (*6*) found that standardized incidence ratios were higher for the two comparison cities than for Duluth. Both reports of cancer incidence in the San Francisco area show an increase in rates with asbestos fiber counts (*4,9*). Conforti et al., who tested the standardized incidence ratios for slope and found the increase for "all digestive tract cancers" significant for white males and for white females, inter-

Table 1. Inherent limitations of studies involving ingested asbestos and possible solutions.

Inherent limitations	Approaches
Exposure duration is short compared to cancer's long latency period (*5–8*)	Increase observation period Maintain surveillance over time
Exposure levels are often low, particularly for asbestos-cement pipe (*7, 8, 12*)	Select study population from high exposure area Increase sample size
Exposure to individuals is not usually assessed in geographical correlation studies (*4–12*)	Design study to examine individual exposures
Probability of outcome is low, requiring extremely large samples	Design study to focus on high-risk groups Use case/control study design to reduce required sample size
Confounding factors exist and can bias results	Control by: stratification or mathematical modeling or restriction of study design to certain areas or individuals

Table 2. Summary of studies of cancer risk in relation to asbestos in water supply by site of neoplasm.[a]

		Site of neoplasm[b]							
Area	Reference	Esophagus	Small intestine	Colon	Rectum	Stomach	Pancreas	Lungs	Peritoneum
Duluth, MN	Mason et al. (5)	OO	—	OO	MF	MF	OF	—	—
	Levy et al. (6)	OO	OO	OO	OO	MO	MF	—	OO
Quebec	Wigle (10)	—	—	OO	OO	MO	OF	MO	—
Connecticut	Harrington et al. (7)	—	—	OO	OO	OO	—	—	—
	Meigs et al. (8)	—	—	OO	OO	OO	B	OO	—
California	Conforti et al. (9)	MF	OO	OO	OO	MF	MF	OO	OF
	Kanerek et al. (4)[c]								
Washington	Polissar et al. (11)	OO	MF	OO	OO	OO	OO	OO	—
Florida	Millette et al. (12)	—	—	—	—	—	OO	OO	—

[a]Adapted from Polissar et al. (11).
[b]M, association in males; F, association in females; B, association in both sexes combined; O, no association; —, not studied.
[c]Conforti et al. (9) update these results.

Table 3. Summary of studies that assessed the association between all digestive cancers and exposure to asbestos.

Area	Reference	All digestive (ICD 150–158)[a,b]	Digestive tract (ICD 150–154)	Digestive-related organs (ICD 155–158)
Duluth, MN	Mason et al. (5)	—	OO	—
Duluth, MN	Levy et al. (6)	OO	—	—
California	Conforti et al. (9)	MF	MF	MO
Florida	Millette et al. (12)	OO	—	—

[a]Code as in eighth revision. International Classification Disease, 1968 (16). 150–154: esophagus, stomach, small intestine, colon, rectum. 155–158: liver, gallbladder, pancreas, retroperitoneum.
[b]M, association in males; F, association in females; O, no association; —, not studied.

preted the findings as indicating a positive gradient with asbestos levels (9). These increases were consistent when stratified on education and income to control for the potential confounding effect of socioeconomic status.

The inconsistent results across these ingestion studies can be interpreted by examining some factors that affect the ability of a study to detect an association. Factors common to nearly all of these studies include lack of control for confounding risk factors, possible misclassification of exposure due to migration, and lack of assessment of variability in daily water source. These factors are inherent to geographic correlation studies using residence as a surrogate for exposure. Factors that vary among the studies and affect the power of the study to detect an increase include the sample size, frequency of outcome, exposure level, duration of the observation period, and duration of the exposure.

Table 4 shows some of these factors for each of the six areas covered by these nine studies. The Canadian study was seriously biased by the confounding effect of occupational exposures (10). In Minnesota, Florida, and Connecticut, duration is barely equal to the suspected 20- to 40-yr latency period for these cancers (13). Exposure levels are low in Connecticut and Florida, thus weakening the association. A lack of association in these geographic areas could be attributed to the inability of those studies to detect low risks. The hi exposure levels and longer duration in Puget Sound and California suggest that the size of the association would be greater in these areas and therefore more easily detectable.

The ability of a study to detect the risk estimated by the AWQC can be assessed by comparing the estimated excess risk with some measure of the ability of the study to detect this risk. The increased risk expected due to asbestos exposure can be estimated by the number of excess cancer cases predicted by the risk assessment in the AWQC. Several assumptions are required to relate the AWQC-estimated 1×10^{-5} lifetime excess risk for 0.3 MFL to the yearly incidence rate. On assuming that all ages are equally at risk and that the dose-response relationship is linear, an approximation of the excess cancer risk per year can be calculated for the California area as follows:

$$\frac{1 \text{ cancer}}{10^6 \text{ persons} \times 70\text{-yr exposure}} \times \frac{20 \text{ MFL}}{0.3 \text{ MFL}} = 1 \times 10^{-5}$$

where 20 MFL is the middle value of the high

Table 4. Characteristics of the epidemiologic studies that affect the power to detect an association.

Study area (reference)	Duration of exposure, yr	Population exposed	Probable exposure level	Estimate of maximum excess cancer per year[a,b]
Duluth, MN (*6*)	≈16–17	100,000	1–30 MFL	—
Connecticut (*7, 8*)	≈19	576,000	<0.1–0.7 MFL	—
Bay Area, CA (*4, 9*)	>40	1,000,000	4–36 MFL	1×10^{-5}
Puget Sound, WA (*11*)	>50	78,000	200 MFL	10×10^{-5}
Florida (*12*)	>25	46,000	<1–10 MFL (AC pipe)	—
	>25	87,000		
Quebec (*10*)	>50	140,000	80–220 MFL	—[c]

[a]Based on AWQCD estimate of 1×10^{-5} lifetime excess risk of gastrointestinal cancer and peritoneal mesothelioma for 0.3 MFL (1 MFL = 3.33×10^{-5} excess cancers) and on assumptions described in the text.

[b]Calculated only for areas with potential for lifetime exposure at time of study: [1 cancer/(100,000 persons/70 yr)] (20 MFL/0.3 MFL) ≈ 1×10^{-5}.

[c]Results biased by occupational exposures in the exposed group.

exposure group. A similar extrapolation for the Puget Sound, using 200 MFL, estimates an excess rate of GI cancers of 10×10^{-5}. Assuming a background incidence rate of 90/100,000 (0.0009) for California and 75/100,000 for Puget Sound for these cancers, the estimated relative risk for exposed groups is 1.01 for California and 1.1 for Puget Sound. There are two other areas of uncertainty in these estimates. First, the average age for the 1973 San Francisco–Oakland population up to age 70 is 31 yr, representing average exposure duration, whereas the AWQC is based on 70 yr exposure. This may understate the risk. Alternatively, using incidence instead of mortality may overstate the risk because the incidence rate for GI cancers is approximately twice the mortality rate.

The ability of a study to detect a given risk is its power. It is not appropriate to estimate power for ecological study designs on the basis of sample size alone because of the unquantifiable biases introduced by misclassification. It is possible, however, to determine whether these studies would be at least large enough to detect a difference in the absence of systematic biases by comparing them to cohort studies of similar size for which power can be estimated. A comparison will be made with studies of cohort design because analyses in these studies focus on exposed and unexposed groups (*14*). However, this exercise can tell us only whether a study is too small; it cannot help to determine if the sample size is adequate.

Sample size tables (*14*) show that for an outcome of 0.001, slightly higher than the incidence of all GI cancers, a cohort sample the size of the California study would have an 80% probability of detecting a relative risk of 1.1 at $\alpha = 0.05$. A cohort sample of the size of the Puget Sound study would have an 80% probability of detecting a relative risk of 1.5 at $\alpha = 0.05$. The Puget Sound study reported the results for single sites: a cohort study this size could detect a relative risk of 4 for pancreatic or stomach cancer. If the risk for these exposures is similar to that predicted by the AWQC, and if the assumptions apply, then these correlation studies would probably be too small to detect the expected risk.

While the study in Puget Sound (*11*) was interpreted to be negative, the 6-yr study in California (*9*) suggests a positive association of cancer of the digestive system with ingestion of asbestos. This study need not be ignored simply because less powerful studies do not verify this endpoint. While the multiple statistical comparisons and the ecological design introduce biases that reduce the power, the high significance level of the test for trend suggests some association with asbestos. The increase in "all digestive cancers" was significant with a low relative risk (<1.1) and was associated with the high exposure census tracts (4–36 MFL). This study suggests that any association between GI cancers and asbestos exposure may be limited to specific sites such as the stomach and pancreas. These are relatively rare cancer sites in humans and require large sample sizes to detect an increase. Polissar et al. (*11*) compared all of the studies and concluded that results for pancreatic cancer were the least likely to be due to chance (see Table 2). The case-control study by Polissar et al. in the Puget Sound area presented at this workshop (*15*) offers an opportunity for similar analyses for specific sites. The minimum risk that could be detected was under 2.0 for each sex for the following grouped or single sites: digestive system, colon, respiratory tract, and lungs.

Discussion

The observation that geographic correlation studies of populations exposed to ingested asbestos detected little evidence for an increased cancer risk must be reevaluated in view of the considerations affecting the power of any study. Several of the studies were insensitive due to low exposure levels and short exposure duration. The potential for misclassification in the ecological study design makes it inappropriate to assess power directly, yet negative studies should be interpreted in terms of the risk each has the ability to detect. The maximum sensitivity that could be expected from the potentially valid studies, those performed in California (*4,9*) and Puget Sound (*11*), leads to the conclusion that the ability to detect increased relative risks <2 (100% increase) is questionable. It is possible to summarize these two studies as follows: Little if any risk was detected in studies that were capable only of detecting the increase in GI cancers if it was greater than that predicted by the AWQC.

Summary

This body of evidence, summarized in view of the general uses of epidemiolgoic data in health risk assessment described above, does not offer quantitative data for estimating levels associated with a defined risk. Due to limited statistical power and inherent biases in the study design, clearly safe and clearly unsafe ranges cannot be definitely identified. The results of the study in the Bay Area by Conforti et al. (*9*) suggests, but does not prove, the endpoint derived from the route extrapolation required for the AWQC. These ingestion studies do suggest that the risk is not greater than that estimated from the inhalation studies and may be less. Due to the large number of individuals with potential lifetime exposure to asbestos, even small increases in relative risks are important; therefore, measures to reduce exposure are still indicated.

To resolve the issue of the human health risk of waterborne asbestos, it would be necessary to initiate analytic epidemiologic studies that have greater sensitivity. Examination of available pharmacokinetic and experimental animal data would be useful. If data from animal studies clearly did not support the association between cancer and ingested asbestos, the need for human studies might be circumvented.

The author thanks James R. Millette of the U.S. EPA for suggesting the basis of Table 4, and Dr. Rebecca H. Osborne of the National Institute for Occupational Safety and Health for suggestions.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Marsh, G. Critical review of published epidemiologic studies of ingested asbestos. Environ. Health Perspect. 53: 49–56 (1983).
2. U.S. EPA. Ambient Water Quality Criteria Document for Asbestos EPA Environmental Criteria and Assessment Office, Cincinnati, October 1980, EPA 440/5-8-022.
3. U.S. EPA. Federal Register. 45 (No. 23): 79318–79379 (November 28, 1980).
4. Kanarek, M. S., Conforti, P., Jackson, L., Cooper, R., and Murchio, J. Asbestos in drinking water and cancer incidence in the San Francisco Bay Area. Am. J. Epidemiol. 112: 54–72 (1980).
5. Mason, T. J., McKay, F. W., and Miller, R. W. Asbestos-like fibers in Duluth water supply. J. Am. Med. Assoc. 228: 1019–1020 (1974).
6. Levy, B. S., Sigurdson, E., Mandel, J., Landon, E., and Pearson, J. Investigating possible effects of asbestos in city water: Surveillance of gastrointestinal cancer incidence in Duluth, MN. Am. J. Epidemiol. 103: 362–368 (1976).
7. Harrington, J. M., Craun, G., Miegs, J. W., Landrigan, P. Flannery, J., and Woodhill, R. An investigtion of the use of asbestos cement pipe for public water supply and the incidence of gastrointestinal cancer in Connecticut, 1935–1973. Am. J. Epidemiol. 107: 96–103 (1978).
8. Meigs, J. W., Walter, S., Heston, J., Millette, J., Craun, G., Woodhill, R., and Flannery, J. Asbestos-cement pipe and cancer in Connecticut, 1955–1974. J. Environ. Health 42: 187–191 (1980).
9. Conforti, P. M., Kanarek, M., Jackson, L., Cooper, R., and Murchio, J. Asbestos in drinking water and cancer in the San Francisco Bay Area: 1969–1974 incidence. J. Chron. Dis. 34: 211–224 (1981).
10. Wigle, D. T. Cancer mortality in relation to asbestos in municipal water supplies. Arch. Environ. Health 32: 185–189 (1977).
11. Polissar, L., Severson, R. K., Boatman, E. S., and Thomas, D. B. Cancer incidence in relation to asbestos in drinking water in the Puget Sound region. Am. J. Epidemiol. 116: 314–328 (1982).
12. Millette, J. R., Craun, G. F., Stober, J. A., Kramer, D. F., Tousignaut, H. G., Hildago, E., Duboise, R. L., and Benedict, J. An epidemiologic study of the use of asbestos-cement pipe for the distribution of drinking water in Escambia County, Florida. Environ. Health Perspect. 53: 91–98 (1983).
13. Selikoff, I. J. Lung cancer and mesothelioma during prospective surveillance of 1249 asbestos insulation workers, 1963–1974. Ann. N.Y. Acad. Sci. 271: 448–456 (1976).
14. Schlesselman, J. J. Sample size requirements in cohort and case-control studies of disease. Am. J. Epidemiol. 99: 381–384 (1974).
15. Polissar, L., Severson, R. K., and Boatman, E. S. Cancer risk from asbestos in drinking water: summary of a case-control study in Western Washington. Environ. Health Perspect. 53: 57–60 (1983).
16. International Classification of Diseases, 8th Revision, U.S. DHEW PHS Publ. No. 1693, U.S. Government Printing Office, Washington, DC, 1968.

Environmental Health Perspectives
Vol. 53, pp. 105–106, 1983

Commentary

The San Francisco Bay Epidemiology Studies on Asbestos in Drinking Water and Cancer Incidence: Relationship to Studies in Other Locations and Pointers for Further Research

by Marty S. Kanarek*

I would like to give my views on why the results in the San Francisco Bay studies (*1, 2*) are generally more positive than the other ecological epidemiology studies that relate asbestos in drinking water to the incidence of cancer. Many other people have speculated, and I think it would be very appropriate here for me to do this also.

I see four possible explanations for the generally larger number of positive results from the San Francisco Bay studies as compared with the studies in Puget Sound (*3*), Connecticut (*4, 5*), and other places.

First, it is possible that the San Francisco Bay Area results are spurious or that the epidemiology studies done in other areas lack enough sensitivity to detect a real effect. This seems doubtful, however, considering how much effort has been made by various parties to negate the San Francisco positive results and to no avail. Also, the studies from Duluth (*6*), Connecticut (*4, 5*), Puget Sound (*3*), and San Francisco Bay (*1, 2*) are apparently so well and thoroughly done that it is hard to dismiss any of them as insensitive in design.

The second explanation is one of simple statistical power considerations. The San Francisco Bay Area study used the largest population. Thus, for cancers of low incidence, it had enough power to detect an association, whereas the other studies had too few cases to merit a similar association.

The third explanation concerns differences in fiber amounts, fiber size and the follow-up period. An explanation for the differences found in the West Coast studies could be that longer fibers were absent in Puget Sound as compared to the distribution of fiber lengths in the Bay Area. Likewise, the asbestos counts in Connecticut were low compared with those in the San Francisco Bay Area. As for follow-up, only now have enough years passed in Duluth to approach encompassing the latent period necessary.

The fourth explanation concerns the biological mechanism of asbestos-related carcinogenesis. The San Francisco Bay Area population may be exposed to some cocarcinogen or not exposed to a possible protective factor. This would especially be important if asbestos were not an initiator. Neither the initiator in the San Francisco Area nor the protective factor in the other areas would have to be in drinking water—food would also be a prime candidate.

Practically, what should be our next steps in answering our questions concerning asbestos in drinking water and cancer? Clearly, population-based incidence case-control studies should now be done on stomach, pancreas, and peritoneal cancers because of the findings of all the ecologic studies put together. Remember, a misdiagnosed peritoneal mesothelioma would most likely be called a pancreatic cancer.

In proper epidemiologic progression, we have conducted descriptive studies in order to refine our hypothesis, in this case to select which cancer body sites warrant case-control studies. Incidence, not mortality case-control studies, should now be done because of the complicated exposure histories necessary in this instance to do a proper study. For individuals with newly diagnosed cancer of the pancreas, peritoneum, and stomach,

*Department of Preventive Medicine and The Institute for Environmental Studies, 504 N. Walnut St., University of Wisconsin, Madison, WI 53705.

and for the appropriate control cases, such incidence case-control studies would seek detailed lifestyle and exposure histories, including lifetime residence histories, occupation, drinking water consumption habits, diet (specifically vitamin intake), cigarette smoking and other factors.

An incidence case-control study would be informative concerning any potential etiologic variables or combination of variables on which data could be gathered from cases and controls. Fiber length should be incorporated in the assessment of individual lifetime asbestos ingestion. Given the basic positive results—the presence of longer fibers and the speculation that a cocarcinogen exists or that an inhibiting or detoxifying variable is absent—the San Francisco Bay Area would be a most fruitful location for such a study.

The views and policies presented by the author in this commentary do not necessarily reflect those of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Kanarek, M. S., Conforti, P. M., Jackson, L., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer incidence in The San Francisco Bay area. Am. J. Epidemiol. 112: 54–72 (1980).
2. Conforti, P. M., Kanarek, M. S., Jackson, P. M., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer in the San Francisco Bay Area: 1969–1974 incidence. J. Chronic Dis. 34: 211–224 (1981).
3. Polissar, L., Severson, R. K., Boatman, E. S., and Thomas, D. B. Cancer incidence in relation to asbestos in drinking water in the Puget Sound region. Am. J. Epidemiol. 116: 314–328 (1982).
4. Harrington, J. M., Craun, G. F., Meigs, J. W., Landrigan, P. J., Flannery, J. T., and Woodhull, R. S. An investigation of the use of asbestos cement pipe for public water supply and the incidence of gastrointestinal cancer in Connecticut 1935–1973. Am. J. Epidemiol. 107: 96–103 (1978).
5. Meigs, J. W., Walter, S. D., Millette, J. R., Craun, G. F., Heston, J. F., Woodhull, R. S., and Flannery, J. T. Asbestos cement pipe and cancer in Connecticut, 1955–1974. J. Environ. Health 42: 187–191 (1980).
6. Sigurdson, E. E., Levy, B. S., Mandel, J., McHugh, R., Michienzi, L. J., Jaggar, H., and Pearson, J. Cancer morbidity investigation: lesson from the Duluth study of possible effects of asbestos in drinking water. Environ. Res. 25: 50–65 (1981).

Environmental Health Perspectives
Vol. 53, pp. 107–108, 1983

Commentary

Assessment of Studies on Cancer Risks from Asbestos in Connecticut Drinking Water

by J. Wister Meigs*

Two related studies were conducted in Connecticut to try to assess cancer risks from asbestos in Connecticut public drinking water (*1–3*). Eleven of the state's 169 towns used source waters containing small amounts of asbestos (less than 500,000 fibers/L as delivered to users). In 82 towns, some, but rarely all, of the population received in their homes water delivered through asbestos cement (AC) pipe located in some part(s) of the distribution systems. The total population potentially exposed on a regular basis to such waters was about 600,000, approximately 20% of the state's population. The average possible duration of exposure was about 20 yr but ranged from over 30 to less than 5 yr.

The published studies were correlational. They considered rates for all cancers, including those of the stomach, colon, rectum, pancreas, lungs, urinary bladder, and kidneys. There was no information about residential mobility or possible occupational exposures of persons who were reported to the Connecticut Tumor Registry (CTR) with newly diagnosed cancers. The U.S. EPA report listed individual cases of peritoneal mesothelioma by town of residence at diagnosis. Possible occupational exposures were explored by searching through city directories. No interviews were conducted for either study.

No consistent evidence of a cancer risk from asbestos in water was suggested in any of the Connecticut work. In comparison with correlational studies from other areas, possible exposures of Connecticut residents to asbestos were quite low, generally only a few hundred thousand fibers per liter as compared with 50 million or more in the water supplies of both San Francisco and Everett, Washington. The second Connecticut study (*2*) should have detected a 50% increased risk in total cancer rates in towns with the highest reported asbestos levels versus those with the lowest ($\alpha = 0.05$, $\beta = 0.90$).

The review of peritoneal mesothelioma gave no indication of a relationship with asbestos in water. A study in progress of all mesotheliomas reported to CTR from 1955 to 1977 indicates that occupational exposures, probably largely to chrysotile, have caused pleural, but rarely peritoneal, localization of this disease. Occupational exposure is suggested in 85% of all cases among men. The study provides circumstantial evidence that causes other than asbestos have probably been important determinants of peritoneal and other nonpleural mesotheliomas (*4*). This comprehensive review of Connecticut's mesothelioma experience is consistent with the negative findings of our correlational studies on possible effects of AC pipe on cancer rates (*1–3*).

The collaborators for these studies included water supply and water quality specialists from the State Department of Health Services and U.S. EPA, and the staff of the CTR and the Department of Epidemiology and Public Health at Yale University. The first work also involved the Centers for Disease Control of the U.S. Department of Health and Human Services. There was general agreement among the authors that although asbestos, as found in Connecticut drinking waters, could never be assumed or proven to be absolutely safe, there was no reason to change "current water distribution policies for Connecticut water supplies because of A/C pipe use"(*2*).

The foregoing conclusions were based on several considerations emphasized in discussions with water supply and water quality specialists, but not included in the report. These were as follows. In many cases, potable drinking water from public supplies is a specialized chemical product and will probably be controlled chemically in the future within even narrower limits than at present. These chemical controls are dic-

*Department of Epidemiology, Yale University School of Medicine, 60 College St., New Haven, CT 06510.

tated by the primary requirement that communicable disease agents be excluded from or brought within acceptable limits in water delivered to users. The controls must also deal with possible corrosive effects of the water on both public piping systems and the systems existing in the homes of users. Third, the leaching of asbestos fibers from AC pipe into potable water supplies can be controlled completely by proper practices. Asbestos fibers occurring naturally in source waters can be reduced by filtration to less than 1% of their original levels.

All treatment systems and all piping systems have the potential for introducing undesirable or hazardous substances into potable water supplies. Some of these are either suspected or known carcinogens. Chlorination may produce trihalomethanes. Asbestos could eventually be shown to be carcinogenic in water supplies despite the lack of satisfactory current evidence. Aromatic hydrocarbons used for coating steel pipe are known to be carcinogenic, although quantities released into potable water are extremely small.

In view of the foregoing considerations, the water supply and water quality specialists who collaborated in the Connecticut studies believed that proper control of drinking water quality would offer the most effective strategy to deal with the potential health risks known or suspected in potable water supplies.

Data reported at the U.S. EPA Workshop on October 13 and 14, 1982, appeared to corroborate the conclusions reached in Connecticut, with the single exception of the ecologic study in the San Francisco area. Further statistical analyses of those data presented at the workshop suggested possible nonasbestos-related explanations for the original findings. The case-control study of cancer in relation to asbestos in water in the Seattle-Everett, Washington, area gave the most powerful epidemiologic evidence reported thus far that human cancers have not been shown to be related to asbestos in drinking water. The 100-fold greater asbestos-in-water exposures of residents of Everett compared with those in Connecticut would make it extremely unlikely that measurable numbers of cancers could have been detected in Connecticut, even if future work identifies a cancer risk from asbestos in drinking water.

It appeared that the most important information gap is in methods for measuring asbestos quickly, accurately, and relatively inexpensively in water as well as in air and foods. Suggestions were made for such methods. Epidemiologic studies of asbestos alone should probably not be planned. However, a comprehensive case-control study that considers trihalomethanes and other organic and inorganic materials, as well as asbestos, should be conducted in areas with adequate historical data about water supplies.

The views and policies presented by the author in this commentary do not necessarily reflect those of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Harrington, J. W., Craum, G. F., Meigs, J. W., Landrigan, P. J., Flannery, J. T., and Woodhull, R. S. An investigation of the use of asbestos cement pipe for public water supply and the incidence of gastrointestinal cancer in Connecticut, 1935-73. Am.J. Epidemiol.107: 96–103 (1978).
2. Meigs, J. W., Walter, S. D., Heston, J. F., Millette, J. Craun, G. F., Woodhull, R. S., and Flannery, J. T. Asbestos cement pipe and cancer in Connecticut 1955-1974. J. Environ. Health 42: 187–191 (1980).
3. Meigs. J. W., and Schoenberger, M. D. Peritoneal mesothelioma in Connecticut in relation to water supplies. Final Report, EPA Contract 68-03-1295, July 26, 1978, unpublished.
4. Teta, M. J., Lewinsohn, H. C., Meigs, J. W., Vidone, R. A., Mowad, L. Z., and Flannery, J. T. Mesothelioma in Connecticut 1955–1977: occupational and geographic associations. J. Occup. Med., in press.

Environmental Health Perspectives
Vol. 53, pp. 109–110, 1983

Commentary

Comments on the California Studies

by Robert C. Cooper*

I became interested in asbestos in drinking water supplies in the spring of 1973 when Mr. Jack Murchio, our electron microscopist, pointed out that a number of San Francisco Bay Area reservoirs were situated on geologic formations that are known to contain asbestos, i.e., serpentine rock. Thus we set out to examine the raw drinking water associated with these reservoirs for the presence of asbestiform fibers. This required the development and refinement of a method for the determination of concentration, enumeration, and identification of fibers in water using electron microscopy. The results of our preliminary investigations indicated that fiber counts as high as 2×10^8 fibers/L were present. These results were published in September 1974 (*1*). To my knowledge, this was one of the first reports of the presence of asbestos fibers in drinking water associated with natural geologic formations rather than with asbestos mining or other industrial activities.

The potential public health problem associated with asbestos in water supplies, particularly in light of the 1974 reports from Duluth, MN, seemed obvious. An epidemiologic study appeared feasible, since the San Francisco Bay Area was serviced by a number of water supplies, some from asbestos-rich geologic formations. Tumor morbidity data for the geographic area involved was available through the San Francisco Bay Area Resource for Tumor Epidemiology. Our epidemiologic study was funded in 1976 by the EPA Health Effects Laboratory. Dr. Marty Kanarek (a doctoral student at the time) was the project manager and Mr. Paul Conforti was assigned as project statistician.

The resultant study was subject to all the problems of such projects. The most time-consuming task was to develop water supply information as it related to the 1970 census tracts of the involved counties and to determine whether or not such information reflected 20–30 years of past history. Among the problems we recognized were the insensitivity of the asbestos assay method, the incomplete employment history available from the tumor registry, the lack of smoking and alcohol consumption data, and the problem of population mobility.

As is well known, the final result of our study (*2*) showed a statistically significant correlation between asbestos fiber concentration in drinking water and cancer incidence, particularly in organs of the digestive tract. We were very cautious to claim only a statistical relationship and not one of direct cause and effect. One troublesome result was a statistically significant negative correlation between uterine cancer and asbestos fiber concentration.

The statistical methods used in our study were relatively simple and straightforward. Since the publication of our results, Dr. M. Tarter, at my request, has rigorously reanalyzed all of our data to determine the reality of the observed correlation. The results of this further analysis have generally reconfirmed our earlier observations. The negative correlation between asbestos level and uterine cancer is most likely a statistical artifact due to the method of residual analysis performed in the original study.

A number of critics raised the question of whether population density might be a major cause of the observed positive correlation. As Dr. Tarter reported, our recent analysis of the effect of population density shows no impact on the correlation between asbestos concentration and tumor incidence. However, in the process of this latter analysis, it was seen that the data from San Francisco City and County *per se* has a major effect upon the correlation that is not related to population density. We are presently referring to this effect as the "San Francisco phenomenon." We are now attempting to describe this phenomenon in more detail and to determine if it is independent of asbestos dose.

If upon further study the relationship between

*Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA 94720.

asbestos concentration in drinking water and cancer incidence appears to be independent of the San Francisco phenomenon, more work will be needed to better estimate if asbestos in drinking water causes cancer in those exposed. A well-conceived case-control study would be the most logical next step.

The views and policies presented by the author in this commentary do not necessarily reflect those of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Cooper, R. C., and Murchio, J. C. Preliminary studies of asbestiform fibers in domestic water supplies. In: Proceedings, 5th Environmental Toxicology Conference, USAF, Dayton, Ohio, 1974, pp. 61–73.
2. Kanarek, M., Conforti, P. M., Jackson, L., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer incidence in the San Francisco Bay Area. Am. J. Epidemiol. 112: 54–71 (1980).

Environmental Health Perspectives
Vol. 53, pp. 111–113, 1983

Commentary

Human Cancer Risk from Ingested Asbestos: A Problem of Uncertainty

by William J. Nicholson*

Human Health Effects

Although studies of populations exposed to high concentrations of asbestos fibers in drinking water appear attractive as research opportunities to define an asbestos-related risk, they are unlikely to provide a definitive answer to the question of whether asbestos in drinking water is associated with an elevated risk of malignancy. Consider, for example, a hypothetical 10-yr analysis of the deaths in a city of 1 million persons whose water supply is contaminated with 100 million fibers per liter (f/L) (a gross overestimate of any real situation). From the estimates in the asbestos criteria document (*1*), ingestion of this water over a 70-yr period would give rise to an added risk of death 3.3×10^{-3} per person.

To estimate the number of asbestos-related deaths in this population, using the above risk data, assume that the average residence time of those deceased in the contaminated area is 14 yr and that the distribution of residence times follows an exponential function, exp $\{t/14\}$. This distribution will certainly overestimate residence times compared with actual populations. Nearly half the census tracts in the U.S. EPA-sponsored San Francisco study, for example, showed more than 53% of the residents moving within 5 yr (*2*). Assume also that 7 yr is required for the risk of asbestos malignancy to manifest itself, as is the case for lung cancer. (See Fig. 1 for the expression of the relative risk of lung cancer in insulators.) This would appear to be so for gastrointestinal cancer (Fig. 2), but will lead to significant overestimates of risk for peritoneal mesothelioma because most such tumors do not appear until after 30 yr or more from first exposure. Approximately 100,000 deaths would be available for analysis over a 10-yr period from a population of 1 million persons. Table 1 lists the excess malignancies expected in this population from the above risk for each period of residence. Those alive will also carry a similar lifetime risk as they achieve the same exposure circumstances.

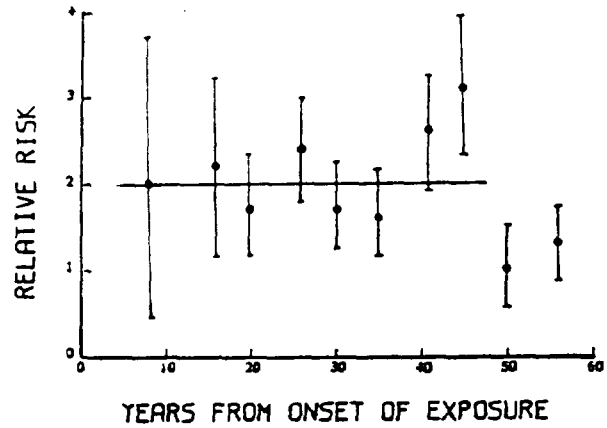


FIGURE 1. Relative risk of lung cancer due to asbestos in insulators.

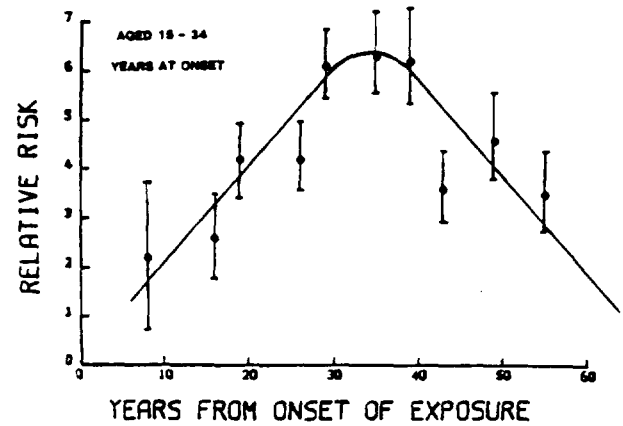


FIGURE 2. Relative risk of gastrointestinal cancer due to asbestos in insulators.

*Environmental Sciences Laboratory, Mount Sinai School of Medicine, City University of New York, New York, NY 10029.

Table 1. Estimated number of excess cancers in 100,000 deceased individuals.

Years exposed	Number exposed (× 1000)	Lifetime risk (× 10^{-4})	Excess deaths
0–6	39.4	0	0.0
7–13	22.7	1.6	3.6
14–20	15.6	4.9	7.6
21–27	8.8	7.2	6.3
28–34	5.3	10.5	5.6
35–41	3.2	14.8	4.7
42–48	2.1	18.1	3.8
49–55	1.2	21.4	2.6
56–62	0.7	24.7	1.7
63–65	0.5	28.0	1.4
70+	0.6	35.0	2.1
		Total	39.5

To simplify the calculations, the excess lifetime risk of the 900,000 alive will be attributed at the time of their deaths and will occur over the ensuing decades. As can be seen, the total additional cancers to be expected in this group is about 40. A cruder, but more direct, calculation is as follows:

$$\underbrace{\left[10^6\right]}_{\text{Population exposed}} \times \underbrace{\left[3.3 \times 10^{-3} \times \frac{14}{70}\right]}_{\text{Average lifetime risk from 14 yr of exposure}} \times \underbrace{\left[\frac{10}{70}\right]}_{\text{Fraction of lifetime for observation}} = 94$$

The more detailed calculation takes better account of the population age distribution (less than 1% die annually, rather than 1/70) and more explicitly accounts for movement out of the area and death elsewhere (within the framework of the assumptions of the model).

If the distribution of excess malignancies parallels that of the working groups on which the risk estimates were made, 17 would be excess gastrointestinal tumors and 23 would be peritoneal mesotheliomas. However, since the risk estimates were based on mortality established by a review of all pathological material, the number of peritoneal mesotheliomas expected on death certificates would be no more than half a dozen. As can be seen, the low yield of excess malignancies expected in such a study would not achieve statistical significance. The expected number of gastrointestinal cancers would be about 4,100 in this population. Since the standard deviation of the expected number is 64, a study as described would show a statistically significant result only if the risk of the fiber concentrations were 7.5 times greater or if the population available for observation were 50 times greater. Thus, the upper limit of risk that can be established by negative studies will be much higher than the risk estimated in the U.S. EPA criteria document *(1)*.

The problem of confounding exposures is also of consequence. The excess projected could be accounted for by the excess mortality from a single shipyard employing 20,000 individuals in the study area (with no such facility in a control area). It is a happenstance coincidence that areas with high concentrations of asbestos in public water supplies also were (and continue to be) major shipbuilding areas. For example, the maximum shipyard employment in the combined San Francisco Bay–Puget Sound areas during World War II exceeded 300,000. Unless shipyard employment is specifically considered, e.g., by careful histories in a case-control analysis, positive studies on ingested water cannot be accepted uncritically.

Animal Studies and Carcinogenic Mechanisms

There is strong evidence that asbestos acts as a promoter for lung cancer in humans and probably in the same way for gastrointestinal cancer as well. In contrast, the fibers appear to act as initiators in the production of mesothelioma. Thus, the analysis of current animal studies should account for these different modes of carcinogenic action. In particular, species otherwise at low risk for gastrointestinal cancer are unlikely to show a significant asbestos effect if asbestos is acting as a promoter. The difference between humans and animals is particularly evident in the history of asbestos lung cancer research. In 1935, it was suggested that bronchogenic carcinoma was related to asbestos *(3)* and, in the forties, this was shown to be so *(4)*. However, this was not demonstrated in animals until 1967. The synergistic effect of cigarette smoke, which acts as an inhibitor in humans, was not present in animal studies.

Control Strategies

The risk of asbestos cancer from ingestion cannot be verified by population studies, and animal experiments involve interspecies comparisons that are uncertain. Nevertheless, human data, albeit from a different exposure route, suggest significant risk for fiber exposures approaching 100 million fibers/L. Standard flocculation and sedimentation techniques can reduce asbestos concentration by about 90%. Such techniques should be adopted in areas with enormous fiber concentrations, since their cost (amortized over time) is relatively modest. Similarly, in aggres-

sive water systems shown to leach asbestos from asbestos-cement (AC) pipes, the water can be chemically treated to reduce the erosion, and new AC pipes can be coated to ensure safety from erosion.

Consider a system having 100 million fibers/L. In a population of 100,000 at exposure equilibrium,

$$\frac{100{,}000 \times 3.3 \times 10^{-3}}{70} = 4.7 \text{ deaths/yr}$$

might occur annually. While the potential deaths would, in general, occur out of the exposure area due to population mobility, their number could certainly justify the necessary system costs.

REFERENCES

1. U.S. Environmental Protection Agency. Water Quality Criteria: asbestos. NTIS, Springfield, VA, EPA 440/5-80-022.
2. Conforti, P. M., Kanarek, M. S., Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer in the San Francisco Bay Area 1969-1974 incidence. J. Chron. Dis. 34: 211–224 (1981).
3. Gloyne, S. R. Two cases of squamous carcinoma of the lung occurring in asbestosis. Tubercule 17: 5–10 (1935).
4. Holleb, H. B., and Angrist, A. Bronchogenic carcinoma in association with pulmonary asbestosis. Am. J. Pathol. 18: 123–131 (1942).
5. Gross, P., and Harley, R. A. Experimental asbestosis: the development of lung cancer in rats with pulmonary deposits of chrysotile asbestos dust. Arch. Environ. Health 15: 343–355 (1967).

Environmental Health Perspectives
Vol. 53, pp. 115–120, 1983

Relative Source Contributions of Diet and Air to Ingested Asbestos Exposure

by James N. Rowe*

Reliable assessments of the relative contributions of diet (food, beverages, and orally administered drugs) and air (inhaled fibers) to total ingested asbestos exposure are not feasible due to the paucity of quantitative data on the subject. Instead, scenarios for both modes of exposure were developed from the limited information available to give crude estimates of ingestion of asbestos from these routes. They suggest that such sources are potentially significant relative to the contribution of asbestos exposure derived from drinking water. Research recommendations are discussed.

The general public has become aware of the potential gastrointestinal exposure to asbestos fibers from drinking water flowing through asbestos cement pipe or from fibers derived from natural sources of asbestos in rivers or reservoirs. However, most people are unaware that diet and air may also contribute significantly to the ingested asbestos exposure. The following is a discussion of the potential relative source contributions of diet, air and drinking water sources to ingested asbestos exposure. Information gaps in this area exist that preclude an accurate measure of the total ingested load of asbestos. The types of studies needed to fill these gaps are identified.

Asbestos in Dietary Materials

Only sparse data are available upon which one can estimate with any assurance the magnitude of asbestos exposure to the gastrointestinal tract from airborne and dietary sources, however, limited information is available for some sources that allow a crude measure of the ingested asbestos load to be calculated. Dietary materials that have been reported to contain, or are likely to contain, asbestos include foods such as vegetable oil, lard, mayonnaise, ketchup, meats (*1–3*) and beverages such as beers, sherries, ports, vermouth and soft drinks (*4–6*). In processed or filtered foods, the contamination is most likely due to the filtration process during which asbestos fibers are released into the processed materials. Other direct or indirect sources of asbestiform minerals in the food industry are in building materials, e.g., cement floor and ceiling tiles, pipe coverings and brake linings of transport vehicles (*7*). In addition, tremolite asbestos is found in talc-coated rice and chewing gum, as well as in oral drugs containing talc as an incipient in compressed tablets, as a dusting powder in capsules, and, less frequently, as a filler in the capsules (*8*).

Contamination by asbestos is so common that many, if not most, foods may contain some asbestos contamination (P. McGrath, FDA, personal communication). Unfortunately, there is presently no established program to examine exhaustively dietary materials for their asbestos content.

Limited, but important, quantitative data on the concentrations of asbestos in ingested sources are given in Table 1. In alcoholic and nonalcoholic beverages, Cunningham and Pontefract (*5*) reported asbestos concentrations ranging from 1.1 to 12.2 × 10^6 fibers/L as measured by the transmission electron microscope (TEM). The asbestos content of a sample of talc-coated rice destined for Japanese consumers was estimated by Merliss (*1*) to contain 3.7 × 10^6 fibers/g as measured through the light optical microscope (LOM). The asbestos concentration in drugs, including three brands of aspirin, has been determined by Nicholson (*9*) to range from 120 to 150 ng/g.

Using this information, a scenario could be constructed along the following lines: John Q. Public is an "average" individual who daily

*Office of Toxic Substances (TS-796), U.S. Environmental Protection Agency, 401 M Street, SW, Washington, DC 20460.

Table 1. Concentration of asbestos in dietary substances.[a]

Dietary substance	No. of observations	Concentration, fibers/L
Beverages		
Beer	4 types	$1.1–6.6 \times 10^6$ (EM)
Sherry	3 types	$2–2.6 \times 10^6$ (EM)
Port	1 type	2.1×10^6 (EM)
Vermouth	2 types	$1.8–11.7 \times 10^6$ (EM)
Soft drinks	4 types	$1.7–12.2 \times 10^6$ (EM)
Talc-coated rice	Not reported	3.7×10^6 fibers/g (LOM)
Drugs (aspirin)	3 brands	120–150 ng/g

[a]Extracted from Office of Toxic Substances data (*10*).

Table 2. Dietary calculations.[a]

Material (portion)	Calculation	Fibers
1 beer (12 oz)	(12 oz) (0.03 L/oz) × [6.6×10^6 fibers/L (EM)]	2.4×10^6 (EM)
Rice (3 oz)	(3 oz) (28 g/oz) [3.7×10^6 fibers/g (LOM)]	310.8×10^6 (LOM)
	310.8×10^6 fibers (LOM) × 1000 EM fibers/LOM f[a]	310.8×10^9 (EM)
	310.8×10^6 fibers (LOM) × 25 EM fibers/LOM fiber[b]	7.8×10^9 (EM)
Aspirin (0.9 g)	(0.9 g) (150 ng/g) [30 fibers (LOM)/ng][c] × 1,000 EM f/LOM f[a]	4.05×10^6 (EM)
		Total daily dose of fibers = 311×10^9 or 7.8×10^9 (EM)
Yearly ingested dose		
With talc		1.14×10^{14} or 2.85×10^{12}
Without talc		2.35×10^9

[a]Data of Winer and Cossett (*11*).
[b]Data of Lynch et al. (*12*).
[c]U.S. EPA data (*13*).

drinks one beer (0.36 L), eats a moderate helping of Japanese-style rice (84 g), and takes three aspirin (0.9 g) to thin his blood (or so he has heard). Using the highest concentration of asbestos reported for beer and aspirin and maximum or minimum conversion factors for the number of EM fibers/LOM fiber (Table 2), we discover that John Q's potential yearly dietary consumption is roughly equivalent to 1.4×10^{14} fibers (maximum conversion factor) or 2.85×10^{12} fibers (minimum conversion factor) in a diet including talc-coated rice. Without talc-coated rice, the yearly ingested dose is 2.35×10^9 fibers (Table 2).

Airborne Asbestos

Estimation of the contribution of airborne asbestos to "secondarily" ingested (swallowed) asbestos exposure is complex. Airborne asbestos enters the body mainly through the nose but also through the mouth. Some fibers may move from these portals of entry to the back of the throat and be swallowed. Much of the inhaled asbestos moves into the tracheobronchial tree to the lungs. Material is cleared from these areas back up the trachea and is then swallowed.

Ingestion of airborne contaminants is dependent upon their patterns of deposition within the nasopharyngeal area and respiratory tract and the rates and pathways for their clearance from the deposition sites to the gastrointestinal tra (*14*).

Generally speaking, inert, insoluble particles deposited in the tracheobronchial area can be cleared by the mucociliary escalator within 1 day via the larynx (*15*). Material deposited in the respiratory epithelium of the alveoli is cleared through nonabsorptive and absorptive processes. Phagocytosis of fibers (approximately 12 μm length or less) by macrophages provides a widely accepted nonabsorptive clearance mechanism for fibers. The phagocytic process is complete within 2 weeks of exposure (*14, 16*). Presumably, macrophages transport asbestos fibers proximally up the respiratory tract for subsequent swallowing (*14*) and passage to the gut. Long asbestos fibers, primarily those > 10 μm length, will remain permanently in the alveoli or penetrate the alveolar wall (*16*).

Estimation of the load of asbestos to the gastrointestinal tract from inhaled fibers must take into account the sites of deposition and their related rates of clearance. The nasal sufaces, nasopharyngeal, oropharyngeal, tracheobronchial

and alveolar regions constitute the major sites of concern. Deposition refers to total fiber concentration deposited in any region of the body following inhalation. For the purposes of this discussion, clearance refers to the processes that result in eventual swallowing of the fibers; e.g., clearance from the nasal mucosa and tracheobronchial regions via mucociliary clearance.

Although no attempt has been made to define accurately the quantitative relationships between inhalation of respirable asbestos fibers (of various size categories) and their subsequent ingestion, there is information that allows for a tentative estimate of ingested or inhaled asbestos, which will be compared to previous quantitative measures developed by other investigators (*17, 18*).

The current analysis relies upon studies performed on non-asbestiform material, namely spherical or amorphous inorganic and organic particles. These studies are reviewed by Lippmann et al. (*14*). In addition, some data are available on asbestos and man-made fibers (*19, 20*).

Exposure scenarios will be considered for two fiber size distributions, i.e., "small" environmental and "large" occupational fibers. Nicholson (*21*) has reported that asbestos fibers in ambient air tend to be very small, with some fibers up to about a micron or so in length, but most are individual fibrils with lengths as short as 0.1 μm and diameters of 0.02–0.05 μm. Although more recently Nicholson has indicated that asbestos fiber sizes from environmental exposure are more equivalent to those from occupational exposures based on monitoring practices in Germany, for the purposes of this discussion we will assume that environmental fiber samples are "small."

The small asbestos particles will be considered as equivalent to more conventional aerosol materials at aerodynamic or linear diameters of 1 μm or less. This particle size range will be deposited primarily in the alveolar region (*14*), with a total deposition of approximately 20% of the inhaled aerosol. The rate of clearance can be estimated from the work of Morgan and Holmes (*20*), who reported that short glass fibers (5 μm length × 1.5 μm diameter) were 80% cleared from rat lungs by 1 yr after deposition.

For the purposes of this paper, it will be assumed that 20% of inhaled asbestos in the ambient air will be deposited and 80% of the deposited asbestos will be cleared. The "average" person is assumed to work for 8 hr/day in this setting and then rests for 16 hr (see Table 3).

In an occupational setting, "large" asbestos fiber lengths are likely to be encountered that have wider diameters than are seen with the "small" environmental exposure fibers. Morgan and Holmes (*20*) have studied the deposition and clearance of glass fibers in rats (1.5 μm diameter × 10 and 60 μm length). They found evidence suggesting 100% deposition of these fibers in the lungs. For UICC chrysotile A and B, crocidolite, amosite, and anthophyllite, which generally have much finer average fiber diameters than glass fibers, the authors reported a 50% deposition rate. The 10 μm glass fibers were cleared to the extent of 70% at 1 yr.

Table 3. Estimated ingested dose following ambient airborne exposure.

	Airborne concentration	Yearly ingested dose (EM fibers)
Office of Toxic Substances estimate[a]	99.8×10^6 fibers/m^3	1.17×10^{11} fibers[c]
Ambient air near Union Carbide Mill and Waste Pile (King City, CA)	1.03×10^6 fibers/m^3	1.20×10^9 fibers[c]
Ambient air near Johns-Manville mill and waste dump (Coalinga, CA, 1973)	593×10^6 fibers/m^3	6.93×10^{11} fibers[c]
Office of Water Regulations and Standards estimates[b]		
Range of 24-hr chrysotile asbestos in ambient air of U.S. cities	100 ng/m^3 (high value) 1 ng/m^3 (low value)	1.1×10^{10} fibers[d] 1.1×10^8 fibers[d]
Ambient air in school	2,000 ng/m^3	2.19×10^{11} fibers[d]
Home of asbestos workers (maximum value)	5,000 ng/m^3	5.48×10^{11} fibers[d]

[a]Source of exposure and selected concentrations derived from OTS data (*10*).
[b]U.S. EPA data (*18*).
[c]Yearly dose calculation: (concentration in air) × (20 m^3 air inhaled/L-day) × (365 days/yr) × (20% deposition) × (80% clearance to gastrointestinal tract). It is assumed that the average person performs 8 hr moderate exercise/day and 16 hr/day at rest in the ambient environment. Daily inhalation volume = [(1450 cc/breath × 8 hr) + (800 cc/breath × 16 hr)] × 15 breath/min × 60 min/hr × m^3/10^6 cc] = 20m^3/d.
[d]Yearly dose calculation: (concentration in air) × (EM fibers/ng: See Table 2) × 10 m^3 air inhaled/day) × (365 days/yr) × (100% deposition and clearance).

In this paper it will be assumed that occupational exposure to "large" asbestos fibers will occur with a 50% deposition efficiency in the respiratory tract and 70% clearance. Occupational exposures are calculated for the work environment only; general ambient exposures outside of the workplace are not included (see Table 4).

Asbestos in Drinking Water

The mean asbestos concentrations (TEM) from a large number of drinking water sources in the United States have been reported by Millette et al. (*23*) to range from below detectable limits (BDL) to over 1 billion fibers/L. Most asbestos concentrations were BDL or not statistically significant (NSS). Only 11% were greater than 10×10^6 f/L. (Note: BDL refers to any result lower than the detectable limit that would be determined if one fiber were counted, using electron microscopy, and the appropriate calculations were made; NSS refers to fiber counts under 5 fibers that give upper and lower confidence limits of ±100%.) Several different analyses of waterborne asbestos have been made and are developed in Table 5.

Relative Sources of Ingested Asbestos

It is widely recognized that asbestos in drinking water may contribute a significant load of the material to the gastrointestinal tract. Using the estimates from the present analysis as well as those developed by other authors, one can see that the annual doses of ingested asbestos for drinking water in the United States could potentially range from 9×10^5 to 4×10^{11} fibers. The surprising finding from preliminary data for dietary and airborne sources indicates that these sources also may pose a significant dose of asbestos to the gastrointestinal tract. It would seem that airborne exposures may deliver from 1.2×10^9 to 9×10^{12} asbestos fibers to the gut, while dietary sources may give 2.4×10^9 to 1.4×10^{14} asbestos fibers yearly. In fact, a comparison of the relative contributions from the various routes of exposure indicates that diet is highest, air is second, and water is the lowest.

It is recognized that these estimates are exceedingly tentative, but they help to point out that sources other than drinking water may deliver

Table 4. Estimated ingested dose following occupational airborne exposures.

	Airborne concentration	Yearly ingested dose (EM fibers)[a]
Office of Toxic Substances estimate[b]		
Fiber release from tearing, crumpling, and cutting asbestos paper	262×10^6 f/m^3	2.30×10^{11} f
Office of Water Regulations and Standards estimates[c]		
Occupational	100,000 ng/m^3	8.76×10^{12} f
American Water Works estimates[d]		
Chrysotile miners and millers (maximum value)	20 mppcf[e]	7.56×10^{11} f
Asbestos production and maintenance workers (minimum value)	2.5 mppcf[e]	1.89×10^{11} f
Chrysotile miners and millers (minimum value)	0.25 mppcf[e]	9.45×10^9 f

[a]Yearly dose estimates, OTS: (concentration in air) × (10 m^3 air inhaled/day) × (250 days/yr) × (50% deposition) × (70% clearance to gastrointestinal tract). It is assumed that the average person performs 8 hr moderate exercise/day during work. No exposure is assumed during leisure hours. Daily inhalation volume = (1450 cc/breath × 8 hr) × 15 breath/min × 60 min/hr × m^3/10^6 cc = 10 m^3/day.

Yearly dose estimate, OWRS: (concentration in air) × (EM fiber/ng: See Table 2) × (8 m^3 air inhaled/day) × (365 day/yr) × (100% deposition and clearance).

Yearly dose estimate, AWWA, for miners and millers: [Concentration (mppcf)] × [2 fiber/cc (LOM)/1 mppcf] × [25 EM fiber/1LOM fiber] × [volume inhaled = 10^3 cc/L × 16 L/min × 420 min/day × 5 days/wk × 50 wk/yr] × [deposition/clearance: 45% (f = 0.5 × 5 μm/EM fiber)].

McDonald (*22*) estimates mppcf = 2 f/cc (LOM); Lynch (*12*) 25 EM fibers/LOM fiber; 1 LOM fiber = 1 × 20 μm, 1 EM fiber = 0.5 × 5 μm; 25 EM fiber per LOM fiber; 1 LOM fiber = approximately 0.000040178 μg; 1 EM fiber = 0.000002645 μg.

Yearly dose estimates, AWWA, for asbestos production and maintenance workers: Same calculations as for miners and millers except 4 fibers/cc (LOM)/L mppcf is used based on Lynch's studies (*12*).

[b]OTS data (*10*).

[c]U.S. EPA data (*18*).

[d]American Water Works Assoc. data (*17*).

[e]mppcf = million particles per ft^3/per year.

Table 5. Estimated ingested dose from drinking water.

	Concentration in water	Yearly ingested dose (EM fibers)[d]
Office of Toxic Substances estimate[a]		
Asbestos-contaminated water (Bishopville, SC)	547×10^6 fibers/L	3.99×10^{11}
Natural contamination: geological source (San Francisco, CA)	130×10^6 fibers/L	9.49×10^{10}
Groundwater (ambient)	3.2×10^5 fibers/L	2.34×10^8
Office of Water Regulations and Standards estimates[b]		
Survey of drinking water	100×10^6 fibers/L (high value)	7.3×10^{10}
	1×10^5 fibers/L (low measured value)	7.3×10^7
American Water Works estimates (municipal water systems)[c]		
Memphis District System	1.70 μg/L	4.69×10^8
Providence District System	0.27 μg/L	7.45×10^7
Saginaw Source	0.0032 μg/L	8.83×10^5

[a,b,c]See Table 4 for sources of exposure and selected concentrations.
[d]Yearly dose estimates, OTS, OWRS: (concentration in water) × 2 L/day) × (365 days/yr).
Yearly dose estimates, AWWA: (concentration in water: μg/gal × gal/L) × (2 L/day) × (EM fibers/μg: see Table 4) × (365 days/yr).

significant amounts of asbestos to the gastrointestinal tract. To the extent that these exposures to the gut may pose health hazards, there may be great reason for trying to define more accurately the contributions of each.

Information Gaps: Dietary Sources

Methods to isolate asbestos from a variety of foods and beverages have been developed (*3, 24*) utilizing SEM and energy dispersive spectroscopy (EDS) analysis. Although these methods appear to provide practical, workable protocols for the potential diagnostic and/or regulatory application, it must be recognized that each food category may present a new set of analytical difficulties that must be resolved. Nevertheless, such methods could be used to examine rigorously the various categories of foods, beverages, or orally administered drugs within the appropriate industries. It is only then that an accurate, quantitative assessment of dietary sources of asbestos can be determined. Estimates of the exposed subpopulation of humans to these materials should also be determined.

Information Gaps: Airborne Sources

The contribution of asbestos to the ingestion exposure from inhalation of airborne fibers has not been critically examined. Experimental and theoretical evaluation of this area would allow accurate dose determinations. Such information could also be applied to any fiber type, both mineral and synthetic, in environmental settings ranging from the occupational to ambient levels. Evaluation requires exposure of animals to a number of fiber size ranges and types with short- and long-term follow-up of the subsequent deposition, retention, and clearance of these fibers. Consideration of the anatomic difference in various species of animals versus humans must also be considered.

Finally, it might be useful to have geographic site-specific data on both ambient water and air concentrations in order to make an accurate comparison of the relative contribution of each source to ingested asbestos exposure.

The author thanks Dr. Richard Hill for his excellent editorial contributions. Special acknowledgement is given to Miss Deanna Kay Dove for her clerical assistance.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Merliss, R. R., Talc-treated rice and Japanese stomach cancer. Science 173: 1141–1142 (1971).
2. Wolff, A. H., and Oehme, F. W. Carcinogenic chemicals in food as an environmental health issue. J. Am. Vet. Med. Assoc. 164: 623–629 (1974).
3. Albright, E. R., Schumacher, D. V., Sweigart, D. S., Stasny, J. T., Husack, C., and Boyer, K. Isolation and identification of asbestos fibers in beverages and foods. Food Technol. 33(11): 69–76 (1979).
4. Biles, B., and Emerson, T. R. Examination of fibers in beer. Nature 219: 93–94 (1968).
5. Cunningham, H. M., and Pontefract, R. D. Asbestos fibers in beverages, drinking water and tissues: their passage

through the intestinal wall and movement through the body. J. AOAC 56 (4): 976–981 (1973).
6. Wehman, H. J., and Plantholt, B. A. Asbestos fibrils in beverages. I. Gin. Bull. Environ. Contam. Toxicol. 11: 267–272 (1974).
7. Huff, J. E. Asbestos: A Perspective, part I, and Overview. Contract Report No. w-7405-eng-26, 1978, pp. 2–17; available from National Technical Information Service, Springfield, VA.
8. Eisenberg, W. V. Inorganic particle content of foods and drugs. Environ. Health Perspect. 9: 183–191 (1974).
9. Nicholson, W. J., Maggiore, C. J., and Selikoff, I. J. Asbestos contamination of parenteral drugs. Science 177: 171–173 (1972).
10. U.S. EPA. Office of Toxic Substances Summary Report of Asbestos Monitoring Data. Versar Inc., August 1982.
11. Winer, A. A., and Cossett, M. The effect of aspect ratio on fiber counts: a preliminary study. In: Health Hazards of Asbestos Exposure (I. J. Selikoff and E. C. Hammond, Eds.), Ann. N.Y. Acad. Sci. 330: 661–672 (1979).
12. Lynch, J. R., Ayer, H. E., and Johnson, D. L. The interrelationship of selected asbestos exposure indices. J. Am. Ind. Hyg. Assoc. 3: 1598–604 (1970).
13. U.S. EPA. Support Document for Final Rule on Friable Asbestos-Containing Materials in School Buildings. Office of Toxic Substances, U.S. EPA, Washington, DC, January 1978, p. 95.
14. Lippman, M., Yeates, D. B., and Albert, R. E. Deposition, retention and clearance of inhaled particles. Brit. J. Ind. Med. 37: 337–362.
15. Albert, R. E., Lippman, M., and Briscoe, W. The characteristics of bronchial clearance in humans and the effects of cigarette smoking. Arch. Environ. Health 18: 738–755 (1969).
16. Morgan, A., Holmes, A., and Talbot, R. J. The fate of inhaled asbestos fibers deposited in the rat lung: a quantitative approach. In: Pulmonary Macrophages and Epithelial Cells (C. L. Sanders, Ed.), National Technical Information Service, Springfield, VA, 1977, pp. 436–450 (CONF-76 0927).
17. American Water Works Association. A study of the problem of asbestos in water. J. Am. Waterworks Assoc. 66(9): Part 2, 1–22 (Sept. 1974).
18. U.S. EPA. Ambient Water Quality Criteria for Asbestos. Office of Water, Regulations and Standards, Washington, DC, EPA 440/5-80-022, October 1980.
19. Evans, J. C., Evans, R. J., Holmes, A., Hounam, R. E., Jones, D. M., Morgan, A., and Walsh, M. Studies on the deposition of inhaled fibrous material in the respiratory tract of the rat and its subsequent clearance using radioactive tracer techniques. 1. UICC Crocidolite Asbestos. Environ. Res. 6: 180–201 (1973).
20. Morgan, A., and Holmes, A. The deposition of man-made mineral fibers in the respiratory tract of the rat, their subsequent clearance, solubility *in vivo* and protein coating. Paper presented at Occupational Health Conference on the Biological Effects of Man-Made Mineral Fibres, Copenhagen, April 20-22, 1982.
21. Nicholson, W. J. Control of Sprayed Asbestos Surfaces in School Buildings: A Feasibility Study. Report to the National Institute of Environmental Health Sciences, June 15, 1978, Contract NO1-ES-7-2113, p. 9.
22. McDonald, J. C. Asbestos in chrysotile mines and mills. Paper presented at Conference on the Biological Effects of Asbestos, International Agency for Research on Cancer, Lyon, France, October 1972, paper no. 23.
23. Millette, J. R., Clark, P. J., and Pansing, M. F. Exposure to Asbestos from Drinking Water in the United States. Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH, EPA 600/1-79-150, 1979.
24. Stasny, J. T., Husack, C., Albright, F. R., Schumacher, D. V., Sweigart, D. S., and Boyer, K. Development of methods to isolate asbestos from spiked beverages and foods for SEM characterization. Scanning Electron Microsc. 1: 587–595 (1979).

Environmental Health Perspectives
Vol. 53, pp. 121–130, 1983

Review of Published Studies on Gut Penetration by Ingested Asbestos Fibers

by Philip M. Cook*

During the 1970s, potential health risks associated with exposure to asbestos in drinking water became a national concern. One of the key questions that arose from debate over whether ingestion of mineral fibers could result in increased gastrointestinal cancer risk was whether fibers can penetrate the gastrointestinal mucosa and thus have some chance of residing in tissue. It is likely that such movement of a large number of fibers is a necessary precursor for carcinogenesis following ingestion of asbestos. Studies of the potential for fiber accumulation in tissues and body fluids following introduction of asbestos to the alimentary canal have provided seemingly contradictory observations. This review, which places particular emphasis on the impact of experimental and analytical limitations on the evidential strengths of each study, indicates the likelihood that a very small fraction of ingested microscopic asbestos fibers penetrates the gastrointestinal mucosa. A reliable estimate of the magnitude of long-term fiber retention in tissues as a consequence of chronic human ingestion of asbestos cannot be made at this time.

During the 1970s, potential health risks associated with exposure to mineral fibers in drinking water became a national concern. This concern developed primarily because of reports of some drinking water fiber concentrations in the range of 10^6–10^9 fibers/L (*1*) and published observations of increased risk of gastrointestinal and peritoneal cancer among asbestos workers (*2*). Inhalation of asbestos dust is accompanied by ingestion of many fibers cleared from the respiratory tract by mucociliary action. Thus a key question in the evaluation of cancer risks associated with the ingestion of asbestos involves the extent to which microscopic fibers, under normal alimentary canal conditions, can migrate through the gastrointestinal mucosa. Such movement of fibers could enable their residence in the bowel wall or, following hematogenous or lymphatic transport, the peritoneum and tissues at other sites. It is likely that such a movement of a large number of fibers is a necessary precursor for carcinogensis following ingestion of asbestos.

This paper will evaluate the published evidence for and against gut penetration and tissue accumulation of ingested mineral fibers. Particular attention will be paid to the influences of analytical techniques employed, fiber properties, and physiological factors on the capability of each investigation to provide useful information about fiber penetration. Most of the work on this question involves microscopic examination of tissues or body fluids for the presence of fibers as a consequence of a known ingestion exposure. Many of these exposures involve controlled laboratory tests with rodents, but a number of human environmental exposures have also been investigated.

Gut Penetration by Durable Particles Other Than Mineral or Synthetic Fibers

Studies involving ingestion of particles other than asbestos, mineral, or synthetic fibers provide some background information generally in support of the movement of a variety of durable particles through the gastrointestinal mucosa as at least an occasional event. Evidence for human intestinal uptake of particles as large as 75 μm is provided by the observation of starch granules in

*Environmental Research Laboratory-Duluth, U.S. Environmental Protection Agency, 6201 Congdon Boulevard, MN 55804.

blood minutes after ingestion (*3*). Volkheimer reported that sleep, smoking, and caffeine increase the number of starch particles in the blood. Dyed cellulose particles were also identified in human blood and urine following ingestion of specially stained food (*4*). The cellulose fibers were found in urine several weeks after ingestion.

Various sizes of latex spheres have been studied. Latex particles of 0.22 μm diameter were reported to migrate from rat stomachs to lymphatics of the gastrointestinal mucosa and also to liver and kidney tissues (*5*). Mice that drank water suspensions of 2-μm diameter latex spheres for 2 months were found to have latex particles in macrophages in intestinal Peyer's patches (*6*). LeFevre et al. (*7*) later reported that mice that ingested 5.1-μm latex spheres had demonstrable accumulations of the particles in intestinal Peyer's patches, lungs, and mesenteric lymph nodes. The frequency of penetration was very small, and evidence for intestinal uptake of very large 15.8-μm latex spheres was not found. Although the difference in size may explain the absence of 15.8-μm sphere in tissues, a 50-fold lower number of large particles ingested appears to have created a corresponding loss of sensitivity for detection of the large particles by optical microscopy.

LeFevre et al. (*7*) did not find evidence for passage of large latex spheres from the intestinal lumen to the portal and peripheral blood as reported for larger starch granules by Volkheimer (*3*). The finding of some particles in mesenteric lymph nodes with a relative absence in the liver was offered as evidence for the restriction of transported particles to the lymphatic system. Most particles that penetrate the Peyer's patch epithelium are sequestered in macrophages and may be transported back into the intestinal lumen without further transport into the body (*7*).

Penetration of mouse intestine by 20- to 50-nm diameter carbon particles occurs almost exclusively through the epithelium covering Peyer's patches (*8*). India ink and ferritin microparticles are rapidly transported from the intestinal lumen by means of pinocytosis observed by electron microscopy of mouse Peyer's patch epithelial cells (*9*). Horseradish peroxidase uptake has been similarly described (*10*). Carbon particles from a 0.5% India ink suspension (20–25 nm diameter) were observed to pass almost exclusively through the posterior intestine of the Amazon molly, *Poecilia formosa* (*11*). Peyer's patches are apparently absent from the intestine of lower vertebrates. Intraperitoneal injection resulted in carbon particle accumulation in the heart, mesentery, and especially the head kidney of the fish.

A final observation of apparent passage of relatively large particles through the intestinal mucosa is provided by the presence of opal-phytoliths in digests of lymph nodes (*12*), and kidneys (*13*) of sheep that are thought to ingest up to 20 g of such particles with a daily intake of plant material.

As with many studies of ingested asbestos fibers, insufficient information is available concerning numbers of particles in nonasbestos exposures and particle detection limits achieved for the tissue preparation and microscope procedures employed to allow a comparison of the observations summarized above. In some regards, attempts to determine if ingested asbestos fibers penetrate the gastrointestinal mucosa provide better test of durable particulate behavior in general. Fiber exposures can provide large numbers of particles available for penetration and the distinctive shapes and elemental compositions of mineral fibers allow better positive identification of the particles in the tissue milieu. On the other hand, the small size of most mineral fibers requires electron microscopic observation of tissues and consequent restrictions on the volume of tissue capable of being searched for fibers.

Experimental Factors to Consider in Evaluating Studies of Fiber Penetration of Tissues

Examination of tissues or body fluids with optical or electron microscopic techniques for identification of mineral fibers can be accomplished either by examination of thin tissue sections or bulk sample residues prepared by removal of carbonaceous and soluble material to concentrate inorganic particles. Thin-section examination provides information about the location of particle contaminants with respect to cells but is very insensitive for finding microscopic particles because of the very small volume of tissue present in a microscope field of view. A multitude of biological, analytical, mineralogical, and kinetic factors must be considered in order to evaluate the significance of a particular finding of the presence or absence of fibers in a sample.

Exposure

Important characteristics of the exposure dose include the size and number of fibers available for penetration of the gastrointestinal mucosa. Use of very large fibers or preparations in which fibers occur in large bundles or clumps for animal exposures may provide few particles capable of penetration. The ability to detect the passage of in-

gested fibers to tissues or body fluids is increased by the ease with which the fibers can be identified microscopically. Durable, easily identified fibers, which are unlikely to be present through contamination of the samples or as a result of another exposure route (inhalation), are especially good tracers for ingested particle studies.

Asbestos and other mineral fibers possess sufficient durability to pass through the acid conditions of the stomach and penetrate the intestinal wall without extensive dissolution. Recent work demonstrates, however, that mineral fibers do differ considerably in their long-term resistance to dissolution while residing in the lungs or in acid solutions (*14*). Alteration of fiber sizes and shapes and splitting of some fibers to increase the number of fibers in tissue can occur during the dissolution process (*14*). Thus it is possible that the dissolution of less durable fibers following penetration of the gut may remove them prior to examination of tissues, whereas other types of fibers may undergo little change or be altered to create many thinner fibers. Phagocytosis, transport, and clearance of fibers are thought to be dependent on fiber size with smaller fibers more mobile. Thus, as these time-dependent processes take place, the number and sizes of fibers at any particular tissue site are likely to be changing. The time from last exposure, the number of particles, and the characteristics of the particles can therefore strongly influence the probability that detectable numbers of fibers will reside in tissue as a result of gut penetration.

Biological Factors

Since so little is known about mechanisms for passage through the gastrointestinal mucosa and subsequent movement of microscopic particles, physiological factors that may influence penetration are unknown. The time over which exposure takes place; the impact of massive fiber doses such as used in some animal ingestion studies; the nature of foods or fluids ingested with the fibers; the species of animal exposed and the relative permeability of its gastrointestinal mucosa; the role of mucosal tissue abnormalities; the age, sex, health, and other characteristics of individual exposed animals; dietary or environmental influences on the motility of the gut; and other factors may determine whether fibers are found when tissues from exposed animals are examined.

The selection of tissues or fluids for microscopic analysis is complicated by a number of considerations. The choice of any particular organ or site for analysis ultimately involves guesswork about where particles would travel after passing through the gastrointestinal mucosa, how long they would take to get there, how long they would reside there, and whether they would concentrate selectively in certain components of the organ. The sampling of portions of organs or body fluids is always coupled with the question of how representative the sample is of the whole. Sample selection must also consider the probability that the finding of a particular kind of fiber in the organ can be associated with a particular route of exposure such as ingestion rather than inhalation.

Analytical Factors

Electron microscopic methods for quantitative assessment of fiber concentrations in air and water have in recent years been successfully adapted to allow determinations of fiber concentrations in tissues and biological fluids. Analytical limitations present for air and water samples (*15*) are generally more severe for tissue analysis, particularly when trace concentrations must be measured (*16*). The primary concerns for tissue analysis are analytical sensitivity, accuracy, and prevention of sample contamination.

Sample characteristics, choice of sample preparation method, and choice of microscope type and conditions all determine the sensitivity of the analysis. Tissues from different organs vary greatly in their reaction to procedures for isolating inorganic particles from the organic matrix, so that the particles can be concentrated into smaller areas for microscope observation. Examination of thin sections frequently is incapable of detecting fiber concentrations likely to be present except in the case of lung tissue following high inhalation exposure. Electron microscopy is required to detect most asbestos fibers that generally have diameters less than 0.5 μm when present in tissues. Sensitivity is maximized through digestion and/or low-temperature ashing of large tissue volumes to produce concentrated residues that are examined by transmission electron microscopy (TEM). Scanning electron microscopy (SEM), although capable of sufficiently high magnification, produces images of sample surface features that are more difficult to search for individual asbestos fibers than TEM images.

Evaluation of reports of tissue analyses for fiber concentrations is impossible without knowledge of the detection limit achieved for each analysis. The detection limit is generally expressed as the concentration of fibers equal to the finding of one fiber in the portion of the sample examined.

Reported concentrations must exceed the detection limit by a factor of at least three or four before any degree of statistical significance is achieved.

The presence of the fibers in blank or control samples as a result of contamination obviously raises the number of fibers that must be observed in order to conclude that the fibers actually accumulated in the tissue. Conclusions made regarding the absence of fibers in tissue are weakened when presented without a demonstration of capability to count the fibers in positive control or spiked samples. When the percentage of recovery is found to be low, it should be used to estimate a more realistic detection limit for the analysis.

Some sample preparation techniques can produce large systematic losses of fibers. Other techniques can increase the number of fibers by fiber comminution. Chrysotile asbestos fiber concentrations are the most difficult to estimate because, in addition to difficulty in identification due to extremely thin diameters and the ease of introduction as contamination, these fibers are readily reduced to individual fibrils by surfactants, ultrasound, and other factors associated with sample preparation. Analytical precision is enhanced when sample preparations observed on the microscope contain a uniform distribution of particles. TEM grids produced by the carbon-coated Nuclepore filter method (*16*) are favored by many analysts for achieving uniform particle distributions in which the number of particles observed in replicate preparations by different laboratories is in reasonable agreement (*17*).

Evidence for and against Fiber Penetration

Several studies of tissues from subjects with occupational exposure to asbestos have resulted in reports of fibers in extrapulmonary tissues and fluids. Wyss (*18*) reported fibers observable by optical microscope in urine samples from asbestos workers. Langer (*19*) reported concentrations of asbestos bodies and uncoated fibers in the order lungs >> kidneys > pancreas > liver for asbestos workmen. Whether fibers arrived at these extrapulmonary sites via lung clearance and ingestion or via migration from the lungs and dissemination throughout the body cannot be determined.

The dissemination of fibers throughout the body has been demonstrated for fibers when introduced by injection into the blood (*20*) or subcutaneously (*21*). Occasional passage of fibers through membranes is suggested by the apparent cross-placental transfer of fibers to fetuses in pregnant rats injected through the femoral vein with chrysotile asbestos (*23*). SEM examination of tissues from mice injected intraperitoneally with amosite, crocidolite, or chrysotile asbestos demonstrated the penetration of the mesothelium by the fibers (*23*). Amphibole fibers have also been observed to migrate from the pleural cavity to the interior of the lungs and the kidneys (P. M. Cook, unpublished data).

More than 30 reports of examinations of tissues from animals and human subjects exposed to asbestos through ingestion (without inhalation exposure) were reviewed. Exclusion of multiple reports of the same observations reduced the number of reports to 19. Rather than discuss the experimental details and results reported for each study separately, Table 1 is provided as a summary and comparison of all 19 studies. Much of the information in Table 1 is presented to allow the reader some idea of how each study meets some of the criteria set forth in this review for evaluating studies of fiber penetration of tissues. Each reference should be consulted for a more detailed evaluation of its experimental design, data quality, and evidence for or against fiber penetration of the gastrointestinal mucosa. In many cases, incomplete information is available in these reports for defining analytical sensitivity, significance of sample contamination, fiber recovery efficiency of the sample preparation procedures, etc. The reduction of complex experimental design and results information to a few words that will fit in the table also requires that only the most generally correct characterizations can be present. For example, the microscope technique is listed as SEM where that technique was the primary method for determining fiber concentrations even though some tissue may have been qualitatively examined by TEM. Other relevant information, such as length of time from exposure to tissue collection, is not presented in the table.

Most of the headings in Table 1 are self-explanatory. The plus or minus symbols for "evidence for/against" indicate each author's conclusions with regard to whether the data reported supports fiber penetration. The fiber dose characterization ideally would provide the total number of fibers passing through the digestive tract. Many studies only report the mass of asbestos or percent of the diet. The duration of exposure varies, as shown in Table 1. In many cases, tissues were analyzed from animals at different time intervals following exposure. The variety of tissues analyzed in some cases is quite large. Positive or negative results for each type of tissue are indicated primarily on the basis of each author's

Table 1. Summary of evidence for and against fiber penetration of the gastrointestinal mucosa.

Evidence for/against	Ref.	Year	Species	No. examined	Fiber type	Fiber dose Characterization	Exposure duration	Tissues analyzed	Tissue preparation method[b]	Microscope technique	Detection limit reported	Positive/negative control samples
+	(24)	1965	Rat	3	Chrysotile	No char., 6% diet	3 mos.	Colon (+) Mesenteric nodes (−) Spleen (−)	Thin sections	TEM	No, qualitative	No/no
+	(20, 25)	1973	Rat	5 5 5	Chrysotile Chrysotile Control	9.6×10^9 fibers 94×10^9 fibers No fibers	Stomach injection	Blood (+) Omentum (+) Brain (+) Lung (−) Spleen (−) Heart (−)	Bulk, soluene, cent HTA, drop	TEM	No	No/contaminated
−	(26)	1974	Rat	10 5	Chrysotile Control	No char., 5% diet No fibers	21 mos.	Intestine (−) Mesentery (−) Other organs (−)	Bulk, bleach, centr, wash, drop	TEM	Incomplete	No/contaminated
			Rat	10 10 5	Amosite tailings Control	No char., 400 mg No fibers	Single gavage	Intestine (−) Mesentery (−) Lung (?) Kidney (−)	Bulk, bleach, centr, wash, drop	TEM	No	No/yes
			Rat	10 10 10	Amosite tailings Control	No char., 10, 20% diet No fibers	6 days	Intestine (−) Mesentery (?) Lung (−) Kidney (−)	Bulk, bleach centr, wash drop	TEM	No	No/yes
−	(27)	1976	Rat	2 2 2 2	Chrysotile Crocidolite Amosite Control	UICC 250 mg/week No fibers	1 yr + 1 mo. free from exposure	GI tract (−)	Bulk, HTA, acid, filt.	SEM	Incomplete, semiqualitative	No/yes
+	(28)	1977	Rat	10 10	Chrysotile Control	Fiber size only, 1% diet No fibers	6 weeks	Blood (?) Omentum (+) Lung (+) Kidney (+) Liver ( ) Brain (+)	Bulk, LTA, acid, filt, LTA, drop	TEM	No	No/contaminated (less than 1973)
+	(29)	1977	Rat	5 2	Amosite Control	UICC, 0.1 mg Saline	1 hr in isolated jejunum segment	Epithelial surface penetration (+)	SEM preparation	SEM	Qualitative	No/yes
−	(30)	1977	Human	282	Asbestos bodies	No char.		Lung (+)	Bulk, LTA	OM	Incomplete	Yes/no
+	(31)	1979	Human	8 8	Amphibole Control	10^8 fibers/L in water $<10^6$ fibers/L in water	Variable by subject	Urine (+)	Bulk, filt, LTA, filt, c-coat, wick	TEM	~10 fibers/ml	Yes/yes
+	(32)	1979	Baboon neonate	1 1	Chrysotile Control	ca. 3×10^{13} fibers in milk No fibers	9 day period	Kidney (+) Liver (−) Spleen (−)	Bulk, LTA, filt, LTA, filt, drop	TEM	~80 fibers/mg	No/yes

Table 1. Summary of evidence for and against fiber penetration of the gastrointestinal mucosa.

Evidence for/against	Ref.	Year	Species	No. examined	Fiber type	Fiber dose Characerization	Exposure duration	Tissues analyzed	Tissue preparation method[b]	Microscope technique	Detection limit reported	Positive/negative control samples
+	(33)	1979	Baboon	1	Chrysotile	UICC, 120 kg Pregavage	4 days, gavage	Urine (+)	Bulk, filt, LTA, filt, LTA, drop	TEM	5×10^2 fibers/ml	No/yes
+	(34)	1980	Human	53[c]	Amphibole Chrysotile	10^8 fibers/L, in water None in water	Variable by subject	Liver (+) Lung (+) Jejunum (+)	Bulk, KOH, centr, HTA, drop	TEM	30 fibers/mg	Yes/yes
+	(35)	1980	Rat	5 5 15 8 2	Chrysotile Crocidolite Chrysotile Chrysotile Control	UICC, $\sim 10^{11}$ fibers UICC $\sim 10^{10}$ fibers NIEHS 10^{11}-10^{13} fibers NIEHS 10^{10}-10^{11} fibers No fibers	Single gavage Single gavage Diet <1-12 days Diet <1-12 days	Lymph Fluid (+) Lymph fluid (+) Lymph fluid (+) Lymph fluid (+) Lymph fluid (+)	Bulk, digestion, filt, c-coat, wick	TEM	Incomplete	No/yes
+	(36)	1980	Rat	10 10 6	Chrysotile Cellulose Control	UICC, 10% diet Cellulose fibers No fibers	2 yr	Colon (+) Colon (−) Colon (−)	Thin, 20 um, HTA	TEM	No	No/yes
+	(37)	1980	Baboon Newborn	1 1	Chrysotile Control	UICC, 3×10^{13} fibers No fibers	9 day period	Kidney cortex (+) Lymph nodes (+) Spleen (+) Colon (+) Esophagus (+) Kidney medula (+) Other sites (−)	Bulk, LTA, filt, LTA, drop		~80 fibers/mg	No/yes
+	(38)	1981	Human	34	Chrysotile	None in drinking water	Unknown	Urine (+)[d]	Bulk, filt, LTA, filt, c-coat, wick	TEM	~10 fibers/mL	No/yes
+	(39)	1981	Human	1	Attapulgite	5 g/day	Drug, 6 mos.	Urine (+)	Bulk, undefined	TEM	No	No/yes
−	(40)	1981	Baboon	1 1	Chrysotile + Crocidolite Control	NIEHS, 800 mg NIEHS, 800 mg No fibers	Gavage, 8 times	Urine (−) Blood (−) Tissues (−)	Bulk, filt, LTA, acetone, drop Bulk, KOH, filt, LTA, acetone, drop	TEM	$\sim 4 \times 10^2$ f/ml $\sim 4 \times 10^4$ f/ml 200 f/mg	Yes/yes

Table 1. Summary of evidence for and against fiber penetration of the gastrointestinal mucosa.

Evidence for/against	Ref.	Year	Species	No. examined	Fiber type	Fiber dose Characerization	Exposure duration	Tissues analyzed	Tissue preparation method[b]	Microscope technique	Detection limit reported	Positive/negative control samples
+	*(41)*	1981	Fish	2	Amphibole	ca. 10^6 fibers/L	Lifetime, water and possibly diet	Kidney (+)[e] Liver (+) Muscle (+)	Bulk, LTA, filt, c-coat, wick	TEM	1-10 f/mg	No/yes
				1	Amphibole	ca. 10^6 fibers/L						
				2	Chrysotile	Unknown						
−	*(42)*	1982	Rat	13	Chrysotile	UICC, 250 mg/week	>20 mos.	9 sites (−)[f]	Bulk, LTA, acid, filt.	SEM	Incomplete	No/no
				12	Amosite							
				13	Crocidolite							

[a]Positive or negative for fibers.

[b]Preparation method steps: thin = removal of a thin tissue section for examination; bulk = processing a large volume of tissue to isolate and concentrate the inorganic particles; HTA = high temperature ashing; LTA = low temperature ashing; KOH, bleach, Soluene = chemicals used for digestion of tissue to remove organic matrix; acid = use of jilute acid to dissolve the nonsilicate portion of the ash; centr = centrifugation to isolate particles from suspension; filt = membrane filtration to remove particles from suspension usually prior to TEM grid preparation; c-coat, wick = preparation of a TEM grid by dissolving a piece of carbon-coated membrane filter by solvent wicking action to leave the particles embedded in the carbon film suspended on the grid; drop = preparation of TEM grid by evaporation of a small drop of a particle suspension on a carbon-coated TEM grid.

[c]Samples were pooled as an exposure group (32 subjects exposed to amphibote fibers in drinking water) and a control group (no amphibole fibers in drinking water).

[d]Number of fibers in urine exceeded number in blank for each urine sample.

[e]Amphibole concentrations in replicate preparations of tissues were found to fit a dose-response relationship with concentrations greatest in kidney and very low in muscle tissue.

[f]*Some fibers were found in tissues (no comparison to blank samples) but concluded to be insignificant on the basis of a lack of preferential retention in any specific tissue, particularly the mesenteric* lymphatic tissues. The authors also indicated difficulty in identifying fine fibers (< 0.1 μm diameter) with the SEM.

conclusions. Question marks are entered where a reasonable uncertainty exists as to whether fibers identified in the tissue are from sample contamination or actually resided in the tissue.

The complexities of tissue sample preparation methods are difficult to condense into a short table entry. In Table 1 they are first categorized as "thin" (microtomed tissue section) or "bulk" (large tissue volumes processed to remove the organic matrix and concentrate inorganic particles). Thin-section analyses are invariably too insensitive to allow the observation of trace concentrations of fibers widely disseminated in tissues. For bulk preparations, a few key words have been added to provide some definition of each bulk preparation technique used. This provides some information concerning the quality of sample preparations, chances for contamination, and uniformity of sample distribution on TEM grids. These key words are provided in the order of the basic steps used for sample preparation. A general sequence involves a digestion and/or ashing (high or low temperature), a concentration of the residue suspended in water by centrifugation or filtration, and the preparation of TEM grids by direct transfer of sample from a filter or application of a drop of suspension to the surface of a carbon-coated grid.

The absence of a clear definition of detection limit, as indicated in Table 1 for many studies, makes the reports of negative tissue analyses difficult to evaluate. Since there can be no demonstration of zero fiber concentrations in tissues, the detection limit, often reported as a "less than" value for each sample, is essential for determining if any particular exposure has resulted in significant accumulations of fibers in the animal. Similarly, the absence of blank tissue control samples makes reports of positive tissue fiber concentrations difficult to accept because of the possibility of fiber contamination. Since it is possible during some sample preparation procedures to systematically lose large numbers of fibers or to fail to detect the fibers during microscope examination of the sample, positive tissue control samples (samples with a known trace concentration) are important for demonstrating that fibers, if present in tissues, can be detected quantitatively. Consequently, negative fiber presence studies in which this capability is not demonstrated have limited authority.

Conclusions

It is difficult to conclude on the basis of the studies summarized in Table 1 that asbestos fibers do not cross the intestinal barrier. The possibility of such passage does not depend on the viability of Volkheimer's "persorption" mechanism (*3*). Most asbestos fibers typically are much smaller than starch granules and closer in length to the 2-μm (*6*) and 5.7-μm (*7*) diameter latex spheres observed by LeFevre et al. to penetrate intestinal Peyer's patches. The widths of many asbestos fibers are actually similar to the 0.02- to 0.05-μm diameters of carbon particles observed to penetrate the epithelium covering Peyer's patches (*8*).

Since it is not possible to design an experiment that demonstrates that no asbestos fibers are retained in tissues following ingestion, we must determine the presence or absence of fibers at a level of detection determined by the analytical considerations discussed earlier. Studies summarized in Table 1 that report the presence of fibers in tissue or fluids generally also report the lowest detection limits, and most offer evidence of negligible fiber contamination as a source of false-positive results. The studies that report negative results are fewer in number and either do not define the analytical detection limit, provide incomplete information, or report a less sensitive analysis than studies reporting positive results for similar experiments.

In addition to consideration of detection limits, which are a pure reflection of the amount of tissue examined, the efficiency of the microscopic technique for detecting individual fibers present in the sample preparation may indicate a further decrease in sensitivity. TEM examination of dispersed particulates is most efficient and allows electron diffraction and energy dispersive X-ray spectral identification of each fiber. One study (*42*) used SEM to detect fibers in bulk tissue preparations but observed: "However the working resolution of this instrument was such that it was difficult to be confident of identifying fine fibers (i.e., less than 0.1 μm in diameter) among residues."

Many of the studies summarized in Table 1 involve exposures to chrysotile asbestos. This is appropriate, since chrysotile is the most common fiber contaminant of water, food, and beverages. Chrysotile fibers, however, serve as poorer indicators of fiber penetration and transport to tissues because of their susceptibility to leaching and comminution and their common occurrence as a contaminant added during sample preparation. Amphibole fibers, including amosite and crocidolite asbestos, and attapulgite fibers were also studied and provide strong indications of fiber penetration. Crocidolite fibers were detected in

lymph fluid of rats fed crocidolite in contrast to the finding of chrysotile fibers in the lymph fluid of other rats fed chrysotile (*35*). Attapulgite fibers were found in high concentration in the urine of a human subject known to have ingested a large amount of that mineral (*39*). Attapulgite is unlikely to be present as a result of a contamination introduced during or after sample collection. Finally, urine (*31*) and tissue (*34*) concentrations of amphibole fibers were shown to be well associated with exposure or absence of exposure to the same amphibole fibers in drinking water. These amphibole fibers existed as a mixture of cummingtonite-grunerite and actinolite with a unique range of elemental compositions that strengthened the association between the identity of fibers in the water ingested and the identity of fibers in the urine and tissue samples.

Perhaps more important than the weight of evidence in favor of the probability of some fiber penetration is the question of what fraction of ingested fibers may be involved. Very little information exists for providing an estimate of this fraction. Any estimate is subject to a number of assumptions and qualifications related to the nature of the data used. The study of fiber appearance in lymph fluid following ingestion or gavage of chrysotile or crocidolite in rats resulted in an estimate of a maximum daily passage of fibers to the lymph fluid of 10^{-4} to 10^{-7} times the number of fibers introduced to the stomach. (*35*). Amphibole fiber concentrations in human urine were observed to represent approximately 10^{-3} of the concentration of the same fibers in the drinking water consumed for up to 20 years prior to the sample collections (*31*). Both the lymph fluid and urine measurements provide a limited basis for an estimate because they do not account for all fibers that may move across the gastrointestinal mucosa. The kinetics of fiber transport and elimination are unknown and further complicate attempts to use the limited data for such estimates of fiber penetration. These estimates do, however, indicate the involvement of a very small fraction of ingested fibers in penetration and consequently low probability for significant tissue accumulations and increased risk of cancer.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Millette, J. R., Clark, P. J., and Pansing, M.F. Exposure to Asbestos from Drinking Water in the United States. Environ. Prot. Tech. Series. Health Effects Research Lab, U.S. EPA, Cincinnati, August 1979, EPA-600//1-79-028, 1979.
2. Selikoff, I. J. Mortality experience of insulation workers in the United States and Canada, 1943-1976. Ann. N. Y. Acad. Sci. 330: 91–116 (1979).
3. Volkheimer, G. Passage of particles through the wall of the gastro-intestinal tract. Environ. Health Perspect. 9: 215–225 (1974).
4. Schreiber, G. Ingested dyed cellulose in the blood and urine of man. Arch. Environ. Health 29: 39–42 (1974).
5. Sanders, E., and Ashworth, C. T. A study of particulate intestinal absorption and hepatocellular uptake. Exptl. Cell Res. 22: 137–145 (1961).
6. LeFevre, M. E., Olivo, R., Vanderhoff, J. W., and Joel, D. D. Proc. Soc. Exptl. Biol. Med. 159: 298–302 (1978).
7. LeFevre, M. E., Hancock, D. C., and Joel, D. D. Intestinal barrier to large particulates in mice. J. Toxicol. Environ. Health 6: 691–704 (1980).
8. Joel, D. D., Laissue, J. A., and LeFevre, M. E. Distribution and fate of ingested carbon particles in mice. J. Reticuloendothel. Soc. 24: 477–487 (1978).
9. Backman, D. E., and Cooper, M. D. Pinocytosis by epithelium associated with lymphoid follicles in the bivisa of Fabricus, appendix and Peyer's patches: an electron microscope study. Am. J. Anat. 136: 455–464 (1973).
10. Owen, R. L. Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in normal undistructed mouse intestine: an ultrastructural study. Gastroenterology 66: 189–199 (1974).
11. Woodhead, A. D. Penetration and distribution of carbon particles in a teleost fish, *Poecilia formosa* (Girard), the Amazon molly. J. Fish Biol. 19: 237–242 (1981).
12. Baker, G., and Jones, L. H. P. Opal in the animal body. Nature 189: 682–683 (1961).
13. Nottle, M. C. Plant particles in kidneys and mesenteric lymph nodes of sheep. Aust. Vet. J. 53: 405 (1977).
14. Cook, P. M., Palekar, L. D., and Coffin, D. L. Interpretation of the carcinogenicity of amosite asbestos and ferroactinolite on the basis of retained fiber dose and characteristics in vivo. Toxicol. Lett. 13: 151–158 (1982).
15. Chatfield, E. J. Measurement of asbestos in the workplace and in the general environment. In: Mineral Association of Canada Short Course in Mineralogical Techniques of Asbestos Determination. Mineralogical Association of Canada, Royal Ontario Museum, Toronto, Canada, 1979, pp. 111–163.
16. Cook, P. M. Preparation of extrapulmonary tissues and body fluids for quantitative transmission electron microscope analysis of asbestos and other mineral particle concentrations. Ann. N. Y. Acad. Sci. 330: 711–724 (1979).
17. Chopra, K. S., Beaman, D., and Cook, P. Interlaboratory measurements of the chrysotile asbestos fiber and mass concentrations in water samples. In: Proceedings of the NBS/EPA Asbestos Standards Workshop, National Bureau of Standards, Gaithersburg, MD, October 1-3, 1980, Natl. Bur. Stds. Spec. Pub. 619 (J. Small, and E. Steel, Eds.), 1982, pp. 121–131.
18. Wyss, V. Presenza di aghi di amianto nelle urine di operai esposti al rischio asbestosico. Rass. Med. Ind. 22: 55 (1953).
19. Langer, A. M. Inorganic particles in human tissues and their association with neoplastic disease. Environ. Health Perspect. 9: 229–233 (1974).
20. Cunningham, H. M., and Pontefract, R. D. Asbestos fibers in beverages, drinking water, and tissues: their passage through the intestinal wall and movement through the body. J. Assoc. Off. Anal. Chem. 56: 976–981 (1973).
21. Kanazama, K., Bisbeck, R. L., and Roe, F. J. C. Migration

of asbestos fibers from subcutaneous injection sites in mice. Brit. J. Cancer 24: 96–106 (1970).
22. Cunningham, H. M., and Pontefract, R. D. Placental transfer of asbestos. Nature 249: 177–178 (1974).
23. Winkler, G. C., and Ruttner, J. R. Penetration of asbestos fibers in the peritoneum of mice. A scanning electron microscope study. Expl. Cell Biol. 50: 187–194 (1982).
24. Westlake, G. E., Spjut, J. J., and Smith, M. N. Penetration of colonic mucosa by asbestos particles. Lab. Invest. 14: 2029–2033 (1965).
25 Pontefract, R. D., and Cunningham, H. M. Penetration of asbestos through the digestive tract of rats. Nature 243: 352–353 (1973).
26. Gross, P., Harley, R. A., Swinburne, L. M., Davis, J. M. G., and Greene, W. B. Ingested mineral fibers. Arch. Environ. Health 29: 341–347 (1974).
27. Bolton, R. E., and Davis, J. M. G. The short-term effects of chronic asbestos ingestion in rats. Ann. Occup. Hyg. 19: 121–128 (1976).
28. Cunningham, H. M., Moodie, C. A., Lawrence, G. A., and Pontifract, R. D. Chronic effects of ingested asbestos in rats. Arch. Environ. Contam. Toxicol. 6: 507–513 (1977).
29. Storeygard, A. R., and Brown, A. L. Penetration of the small intestine mucosa by asbestos fibers. Mayo Clin. Proc. 52: 809–812 (1977).
30. Auerbach, O., Hammond, E. C., Selikoff, I. J., Parks, V. R., Kaslow, H. D., and Garfinkel, L. Asbestos bodies in lung parenchyma in relation to ingestion and inhalation of mineral fibers. Environ. Res. 14: 286–304 (1977).
31. Cook, P. M., and Olson, G. F. Ingested mineral fibers: elimination in human urine. Science 204: 195–198 (1979).
32. Patel-Mandlik, K. J., Hallenbeck, W. H., and Millette, J. R. Asbestos fibers: 1. A modified preparation of tissue samples for analysis by electron microscopy. 2. Presence of fibers in tissues of baboon fed chrysotile asbestos. J. Environ. Path. Toxicol. 2: 1385–1395 (1979).
33. Hallenbeck, W. H., and Patel-Mandlik, K. J. Presence of fibers in the urine of a baboon gavaged with chrysotile asbestos. Environ. Res. 20: 335–340 (1979).
34. Carter, R. E., and Taylor, W. F. Identification of a particular amphibole asbestos fiber in tissues of persons exposed to a high oral intake of the mineral. Environ. Res. 21: 85–93 (1980).
35. Sebastien, P., Masse, R., and Bignon, J. Recovery of ingested asbestos fibers from the gastrointestinal lymph in rats. Environ. Res. 22: 201–216 (1980).
36. Donham, K. J., Berg, J. W., Will, L. A., and Leminger, J. R. The effects of long-term ingestion of asbestos on the colon of F344 rats. Cancer 45: 1073–1084 (1980).
37. Patel-Mandlik, K. J., and Millette, J. R. Evidence of migration of ingested asbestos into various baboon organs. Scanning Electron Microsc. 1: 347–354 (1980).
38. Cook, P. M., and Longrie, D. L. Determination of asbestos and other inorganic particle concentrations in urine. I. Electron Microscopy and X-Ray Applications to Environmental Occupational Health Analysis, II (P. A. Russel, Ed.), Ann Arbor Science Publishers, Inc., Ann Arbor, MI, 1980, pp. 219–231.
39. Bignon, J., Sebastien, P., Gaudichet, A., and Jaurand, M. C. Biological effects of attapulgite. In: Biological Effects of Mineral Fibres, IARC Sci. Publ. No. 30, Vol. 1 (J. C. Wagner, Ed.), Lyon, France, 1980, pp.163–189.
40 Hallenbeck, W. H., Markey, D. R., and Dolan, D. G. Analyses of tissue, blood, and urine samples from a baboon gavaged with chrysotile and crocidolite asbestos. Environ. Res. 25: 349–360 (1981).
41. Batterman, A. R., and Cook, P. M. Determination of mineral fiber concentrations in fish tissues. Can. J. Fish. Aquat. Sci. 38: 952–959 (1981).
42. Bolton, R. E., Davis, J. M. G., and Lamb, D. The pathological effects of prolonged asbestos ingestion in rats. Environ. Res. 29: 134–150 (1982).

Environmental Health Perspectives
Vol. 53, pp. 131–139, 1983

Use of Quantitative Analysis of Urine to Assess Exposure to Asbestos Fibers in Drinking Water In The Puget Sound Region

by Edwin S. Boatman,* Tod Merrill,* Angela O'Neill,* Lincoln Polissar† and James R. Millette‡

An earlier epidemiologic and electron microscopy study of drinking water in the Everett area of Washington State indicated large numbers of naturally occurring chrysotile asbestos fibers in the water. The purpose of the present study was to determine whether significant numbers of asbestos fiber could be demonstrated in the urine of donors residing in that area for less than 3 yr and over 20 yr where the tapwater contained about 200 × 10^6 fibers/L. A control group was obtained from Seattle where the tapwater asbestos fiber content was 100 times less. Urine samples, filtered control water, tapwater samples, and additional controls were processed for transmission electron microscopy by the use of the Nuclepore membrane filter-Jaffe wick procedure. Interference by mucos in the urine was reduced by treatment with hydrogen peroxide. Samples were taken over a period of 21 months. At no time during this period did the asbestos content of the urine samples consistently exceed that of the control waters. There was a significant difference ($p < 0.05$) in the asbestos content of urine samples from subjects with < 3 yr residence times versus > 20 yr. Asbestos concentration in urine samples from Everett residents as a whole did not differ significantly from that in samples from Seattle residents. Variable degrees of chrysotile contamination of control water samples and of Nuclepore membrane filters presented a problem. At present, the data are inconclusive but would suggest no relationship between high concentrations of fibers in drinking water and the numbers estimated for voided urine.

Introduction

There are now ample data to indicate that drinking waters taken from cities throughout the United States and Canada contain naturally occurring asbestos minerals (*1–3*). While chrysotile asbestos is the most prevalent form seen, amphibole asbestos is also found either alone or, more commonly, in admixture with the chrysotile variety. Millette et al. (*4*) found concentrations of chrysotile asbestos in drinking water ranging from greater than 1 × 10^6 fibers/L to more than 10 × 10^6 fibers/L in 186 out of 406 cities in the United States, and a study by Chatfield and Dillon (*5*) indicated a similar range of concentrations in 71 locations across Canada. Other fluids, including wines (*6*) and beers (*7*), have also been shown to contain significant numbers of asbestiform fibers. From these and other observations, some obvious points of concern have arisen with respect to what happens to these fibers when imbibed. Do significant numbers penetrate the wall of the intestinal tract? Are they found in other organs of the body and body fluids? What is their effect, if any, of long-term exposure for 20 yr or more? Clearly, although it is easy enough to estimate the probable concentrations taken in during the consumption of water over time (without regard, of course, to the contributions from beverages and food), it is extremely difficult to determine the ratio of fibers retained or excreted. From the work of Cunningham et al. (*8*) on the accumulation of asbestos fibers in the feces of workers exposed to moderate to high industrial

*Department of Environmental Health, University of Washington, Seattle, WA 98195.
†Fred Hutchinson Cancer Research Center, Seattle, WA 98104.
‡Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH 45268.

levels of chrysotile, it appears that around 12–26 × 10^6 fibers/gm of feces (perhaps 12–52 × 10^8 fibers in a 24-hr specimen) may be present. Control subjects yield about 0.4 × 10^6 fibers/g of feces. About 85% of the fibers isolated (irrespective of group) were less than 2.0 μm in length. Unfortunately, there are no data on the probable intake levels.

Although the results from animal experiments and human autopsy material have indicated the passage of ingested chrysotile asbestos fibers through the gastrointestinal wall and their presence in many organs of the body other than lungs, notably omentum, kidneys, urine and brain (*9, 10*), the long-term effects of ingestion of low to moderate numbers of fibers on body-organ function is unclear. Recent work by Hallenbeck et al. (*11*) indicates no evidence that asbestos fibers penetrate the gastrointestinal tract and migrate to various tissues. Contrary to these results, Sebastien et al. (*12*) have evidence that chrysotile and crocidolite asbestos fibers do pass across the wall of the gastrointestinal tract with the passage rate being higher for long fibers than for short ones.

Fibers other than asbestos may be found in the urine and in considerable numbers. In a single case, Bignon et al. (*13*) found attapulgite fibers at a concentration of 300 × 10^6 fibers/L in the urine of a 60-yr-old woman treated with a drug containing attapulgite for 6 months at a dose of 9 g/day orally. The mean length for the fibers was 0.93 μm.

Neoplasmic changes in the lungs due to inhalation of asbestos are well documented, but evidence for a similar causation in other organs is less strong. Studies by Kanarek et al. (*14*) and Conforti (*15*) indicated a positive but low risk for tumors of the digestive tract in relation to imbibed asbestos for a population of the San Francisco Bay Area. Polissar et al. (*16*) studied cancer incidence and mortality with respect to asbestos in drinking water in the Puget Sound region (Washington State). Studying communities with long-term exposures of 20+ yr, the authors found few associations between imbibed asbestos and cancer and concluded that chance was the most likely explanation for the cancer incidents. From these results and those from previous studies, however, they proposed that the pancreas and small intestine were sites that should be included in follow-up studies.

Recently, Cook and Olson (*17*) published findings indicating the presence of significant numbers of amphibole asbestos in urine samples from residents of Duluth, Minnesota, who were drinking unfiltered water derived from Lake Superior that was known to contain, on the average, 30 × 10^6 fibers of amositel/L water. Subsequent epidemiologic studies in Duluth (*18, 19*) revealed no significant increase in the risks of various cancers in the exposed population.

Because of the epidemiologic and drinking water asbestos analysis data accumulated on the cohort groups in the Everett, Puget Sound region (*16*), it was decided to carry out a limited study on a small group of people from this area to ascertain whether or not significant numbers of chrysotile asbestos fibers could be demonstrated in the urine of people residing in that area for more than 20 and whose drinking water contained about 200 10^6 fibers/L. These people would be compared to a control group where the level of chrysotile asbestos in the drinking water averaged 2.0 × 10^6 fibers/L (i.e., the Seattle area, Tolt River supply).

Materials and Methods

Selection of Specimens

In order to obtain urine samples in a precise and consistent fashion, male staff from the Everett Public Health Laboratories were recruited as donors. Personnel from the School of Public Health, University of Washington, were recruited as controls. All subjects were in good health, and none reported any history of kidney disease or occupational exposure to asbestos. Subjects also completed a questionnaire documenting their source of drinking water, personal consumption and other beverages taken regularly. The ages, residence times, and other data of the donors are given in Table 1. Three standard prerinsed 1-qt plastic containers were given to each subject. One container contained 200 mL of 0.1-μm Nuclepore-filtered distilled water (control), which was

Table 1. Asbestos analysis: donor urine.

Donor No.	Age	Years in residence (Everett)	Years at present address	Mucous threads[a]
101	55	24	6	Moderate
102	56	24	24	Moderate
103	62	30	13	Moderate
104	40	30	0.75	Moderate
107	28	1.5	1.25	Moderate
108	25	2.75	0.75	Scanty
109	44	1.5	1	Moderate
501[b]	41	19 (Seattle)	19	Scanty
502[b]	24	12 (Seattle)	2	Moderate
503[b]	—	5 (Bellevue)	5	Moderate
504[b]	25	3 (Bellevue)	3	Moderate

[a]Qualitative estimates by Nomarski interference optics.
[b]Control.

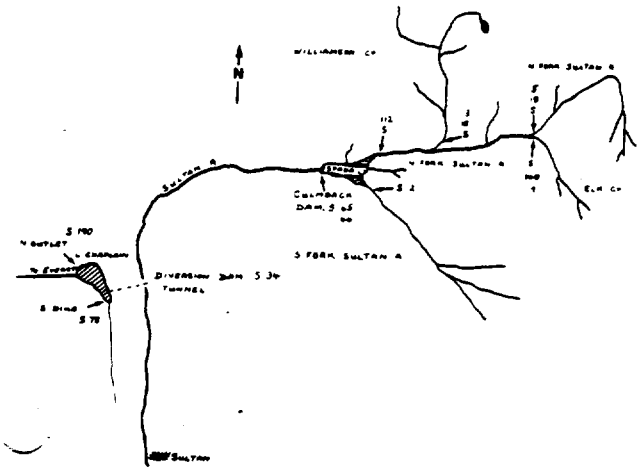


FIGURE 1. Sketch of Sultan River watershed showing sampling points and asbestos fiber concentrations. Not to scale.

simply opened and closed at the time of urination into the second and empty container. The third container was to be filled with tapwater from the place of residence. All urine samples were freshly voided, early-morning specimens that were delivered to the testing laboratory on the same day. Drinking water in the Everett area is supplied by the Sultan River system (Fig. 1).

The voided volume and specific gravity were recorded, an aliquot (10 mL) of each urine sample was centrifuged at 1,500 rpm for 10 min, and the deposit was mounted in a drop of methylene blue and examined by Nomarski interference optics for the presence of epithelial and other cell types, casts, and mucous strands. The presence of mucus was a consistent feature of the urine samples and, accordingly, presented a problem during the filtration procedure. After trials using various chemicals and enzymes (e.g., trypsin, urea, pronase, ozone, *N*-acetyl L-lysine, and hydrogen peroxide, it was found that the H_2O_2 method of Cook and Olson (*17*) was the most effective in disaggregating the mucous strands. Consequently, the volume remaining with each urine sample was mixed with equal volumes of 30% H_2O_2 and allowed to react at 40°C for 20 hr with intermittent shaking on a rotary shaker for the first hour. The maximum possible volume of urine (from 25 to 100, 50 mL being the general case) was filtered through 0.2-μm filters with the minimum of negative pressure. Prefiltered control water samples (50 mL) were filtered through a 47-mm diameter, 0.2-μm pore size Nuclepore filter, and tapwater samples through a 47-mm diameter, 0.1-μm Nuclepore filter, each supported by a 2.0-μm pore size Millipore backing filter. Although all tap water samples were collected, not all were analyzed because of the consistently high levels of asbestos established. Low temperature ashing as in the Cook and Olson procedure was not used, and, after drying, an equatorial strip was cut from each Nuclepore filter, attached to a glass slide, and coated with carbon by rotation in a vacuum evaporator; then, small portions of the coated filters (2 mm^2) were placed on 200-mesh copper-rhodium electron microscopy (EM) grids (Maxtaform, Fullham, NY). The grids were exposed to chloroform vapors in accordance with the modified Jaffe wick procedure of Chatfield et al. (*20*) and the filter matrix dissolved. After dissolution of the filter, the grid containing the particulates retained by the surface carbon film was observed by a JEOL 100S transmission electron microscope (TEM) operating at 100 kV at a magnification of 21,000×. The tasks of preparation of the samples and the subsequent fiber analysis were distributed between two experienced observers.

Controls

Additional controls, consisting of filtered H_2O_2 solutions, urine samples, "spiked" with a predetermined number of chrysotile asbestos fibers, and urine and control water filtrations through Nuclepore membranes of different batch numbers of 0.1- or 0.2-μm pore size, were also set up. Nuclepore membranes of different lot numbers were ashed to determine their endogenous asbestos fiber content.

Sample Analysis

Twenty grid openings selected randomly from three or four grids of each sample were examined. Particulates suggestive of asbestos fibers were rated according to aspect ratio, morphology, and crystal structure (selected area electron diffraction, or SAED). In some instances, energy-dispersive X-ray analysis was also attempted. The concentrations of fibers in millions per liter were calculated by use of the standard formula of Anderson and Long (*21*).

Detectable Limits

The sensitivity of the asbestos analysis method is dependent upon the pore size of the filter used, the volume of urine or water filterable, the presence of interfering particulates (e.g., fragments of diatoms, residues of mucus, and other debris), and the number of grid openings searched (*4*). In our analyses, we obtained limits of detection ranging from 0.07 to 0.3 × 10^6 fibers/L.

It is important to be aware that the areas of individual grid openings measured by light microscopy of 200-mesh microscope grids may vary considerably from batch to batch. In one sequence of measurements from different batches, we obtained readings ranging from 7995 to 9988 μm^2 for a single grid opening.

Results

As shown in Table 1, it is seen that, of the seven donors from the Everett area, four had spent more than 24 yr in the area, and the remaining three, less than 3 yr. All were exposed, however, to high numbers of chrysotile asbestos fibers in their drinking water (Table 2). The numbers of fibers per liter calculated for urine, control water and drinking water are shown in Table 2, with summary statistics in Table 3. Although the concentrations of fibers in the urine varied throughout the 21-month sampling period, none was consistently and significantly higher than the concentrations of fibers estimated for the control waters. There was, however, a significant difference (p = 0.05) between the counts of fibers in the urine of donors with less than 3 yr residence time (Everett area) and the urine of donors with 24+ yr residence time. Note, however, that the fiber count in the urine of donor No. 102 was higher than that of all others of the Everett group; otherwise, the mean concentrations for long-term versus short-term exposure were similar. The concentration of fibers in the urine of Everett donors combined (0.97×10^6 fibers/L) was not significantly different from the concentration for Seattle/Bellevue control donors, even though the numbers of fibers in the drinking water of the latter were at le[illegible] 100 times less. The results of various cont[illegible] samples processed with different batches of Nuclepore membrane filters are shown in Table 4. Overall, use of either of the two batches of 0.2-μm Nuclepore membranes in the filtration step appears to yield about a fourfold increase in number of fibers present compared to filtration through a 0.1-μm pore size membrane. It should be noted that a 2.0-μm Nuclepore membrane is twice as

Table 2. Asbestos analysis: chrysotile fibers in urine, control water, tapwater.

Location	No.	Years in residence	Chrysotile fibers/L ($\times 10^{-6}$)[a] Urine[b]	Control water[b]	Tapwater[b]
Everett	101	24	0.85 ± 1.00	1.63 ± 1.34	230 ± 57
	102	24	2.70 ± 2.47	2.00 ± 1.94	320 ± 254
	103	30	0.68 ± 0.71	1.83 ± 1.07	295 ± 60
	104	30	0.70 ± 0.14	1.50 ± 1.11	297 ± 230
	107	1.5	0.70 ± 0.87	1.30 ± 0.89	235 ± [illegible]
	108	2.8	0.57 ± 0.45	1.27 ± 0.72	259 ± [illegible]
	109	1.5	0.27 ± 0.29	0.93 ± 0.55	383 ± 245
Seattle/Bellevue	501	19	2.03 ± 1.33	1.17 ± 0.15	1.17 ± 0.15
	502	12	0.33 ± 0.25	0.43 ± 0.06	2.70 ± 0.95
	503	5	0.43 ± 0.42	0.77 ± 0.15	3.07 ± 1.00
	504	3	0.50 ± 0.10	1.03 ± 0.25	2.50 ± 0.50

[a]Means of samples from November 1979 to August 1981, ± 1 SD.
[b]Nos. 101-104: sample (n) for urine and control water = 4; for tapwater = 2. Nos. 107-109 and 501-504: sample (n) for all procedures = 3.

Table 3. Asbestos analysis: chrysotile fibers in urine, control water, tapwater; statistical summary.

Group	Years in residence	Chrysotile fibers/L ($\times 10^{-6}$)[a] Urine	Control water	Tapwater
Everett				
Long term 4	27	1.23 ± 1.51 (n = 16)	1.74 ± 1.28 (n = 16)	285 ± 138 (n = 8)
Short term 3	2	0.51 ± 0.55 (n = 9)	1.17 ± 0.66 (n = 9)	278 ± 214 (n = 9)
Seattle/Bellevue 4	9	0.83 ± 0.95 (n = 12)	0.85 ± 0.33 (n = 12)	2.36 ± 0.98 (n = 12)

[a]± 1 SD. t-test; Everett: urine: long term vs. short term. 1-tail test, p = 0.05; control waters, NS (2-tail test); tapwaters, NS (1-tail test). Everett vs. Seattle/Bellevue: urine, NS (1-tail test); control waters and tapwaters, p = 0.01 (2-tail test) and $p < 0.001$ (1-tail test), respectively.

Table 4. Controls.

Sample	Nuclepore membrane		Number of chrysotile fibers
	Pore size	Batch number	$\times 10^6$/L
100 mL glass-distilled water (GWD)	0.1 μm	83B 9B27	0.14
100 mL GDW	0.1	93B 9A31	0.20
100 mL filtered H_2O (0.1 μm) GWD	0.1	83B 9A21	0.14
100 mL filtered H_2O (0.1 μm) GDW	0.2	83N 9A84[a]	0.50
100 mL GDW + H_2O_2	0.1	83B 9A31	0.18
100 mL GDW + H_2O_2	0.2	83N 9A84	0.85
100 mL GDW + H_2O_2	0.2	83B 5F9[a]	0.65
25 mL urine (A) + 25 mL asbestos H_2O + 50 mL H_2O_2	0.2	83B 5F9	5.8
25 mL urine (B) + 25 mL asbestos H_2O + 50 mL H_2O_2	0.2	83B 5F9	4.3
25 mL filtered H_2O 25 mL asbestos H_2O 50 mL H_2O_2	0.2	83B 5F9	8.9

[a]Batch 83N 9A84 gave 21.1 fibers/grid opening after ashing for 2.5 hr at radiofrequency, 50 W; and batch 83B 5F9 gave 6.4.

thick (i.e., 10 μm versus 5 μm thick) as a 0.1-μm pore size membrane. Prefiltration of the glass distilled water and H_2O_2 in tests for asbestos contamination showed no contribution from these sources.

At the bottom of Table 4 are entries for urine samples with an added quantity of water containing a known amount of chrysotile asbestos fibers; these are compared with an asbestos control containing filtered distilled water in place of urine. It is seen that the mean concentrations of fibers calculated for the "spiked" urine (5.0×10^6) are 57% of the asbestos control concentration (8.9×10^6). Both of the spiked urine samples were shown to contain moderate amounts of residual material on the EM grids even after treatment with H_2O_2. It was further calculated that a loss (i.e., embedded in mucous residues) of one fiber/two grid openings would account for the 43% reduction in fiber counts for these urines.

The mean length of fibers found in the urine samples was 0.9 μm with a range of 0.5 to 1.2 μm. In the drinking water, fibers ranged in length from 0.3 to 5.0 μm with a mean of 0.8 μm. Electron diffraction showed 68% of these fibers to be positive for chrysotile. Among fibers counted as asbestos, 26% gave strong SAED patterns, 52% moderate patterns, and the remaining 22% were weak but discernible. About 32% of all fibers examined failed to give a recognizable diffraction pattern and were not counted.

Light microscopy of centrifuged urine deposits nearly always showed residues of mucous threads (Fig. 2) which, following treatment with H_2O_2, were effectively disaggregated (Fig. 3). Scanning electron microscopy (SEM) of the deposit from 10 mL of an untreated urine sample filtered through a 0.2-μm Nuclepore membrane, dried and coated with gold-palladium, showed a homogeneous layer of mucus 1.0 μm thick covering the membrane surface (Fig. 4, arrow). The residue from an H_2O_2-treated and filtered urine sample processed for TEM and showing a single chrysotile asbestos fiber is seen in Figure 5. In this figure, note also the apparent blocking of pore apertures by residual material. (arrow).

Discussion

Drinking water becomes contaminated by asbestos fibers as a result of geologic erosion, pollution (e.g., logging and the building of dams) and the internal erosion of asbestos-cement pipe. The consistently high concentrations of chrysotile fibers of about 200×10^6 fibers/L found in the water supply of Everett, Washington, are unique for this area, but concentrations between 1 and 100×10^6 fibers/L have been found in a number of

FIGURE 2. Light microscopy of urine sediment (10 mL) before treatment with 30% H_2O_2. Note abundance of mucous strands. Nomarski optics. ×200.

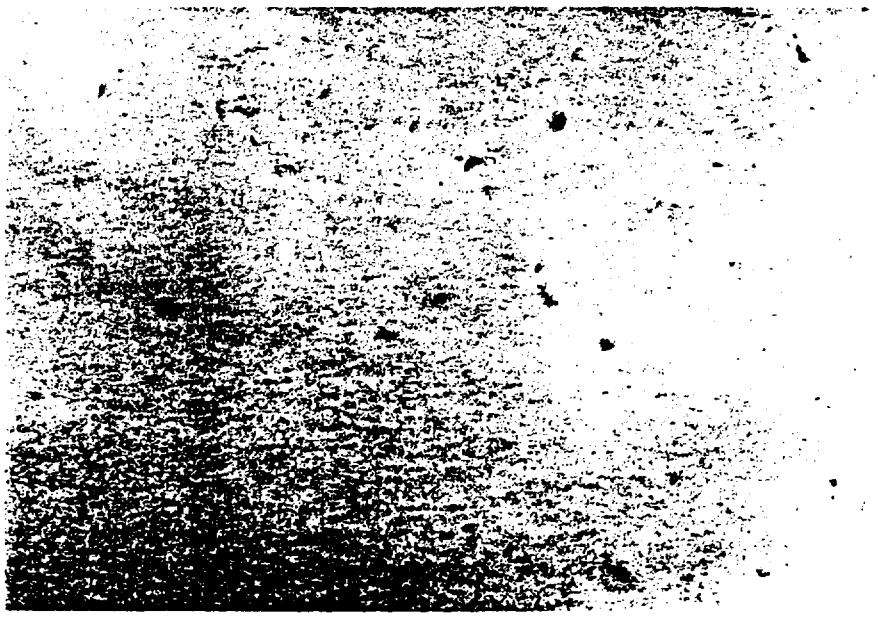


FIGURE 3. Light microscopy of urine sediment after treatment with 30% H_2O_2. Little residue is seen at this level of magnification. Nomarski optics. ×200.

water supplies in California (*4*). Most of this contamination on the West Coast is considered to be due to the erosion of asbestos-bearing rocks aided, at times, by marked fluctuations in the local meteorologic conditions. This combination results in relatively high concentrations of fibers in the water supply throughout the year and is grounds enough for believing that unusually high numbers of fibers may be found in the urine of people drinking this water compared to appropriate controls. It has been variously estimated that approximately 10^{-3} (*17*) to 10^{-4} (*22*) of the ingested fibers find their way into the urine. In the first case, after 2 L water is imbibed, we would

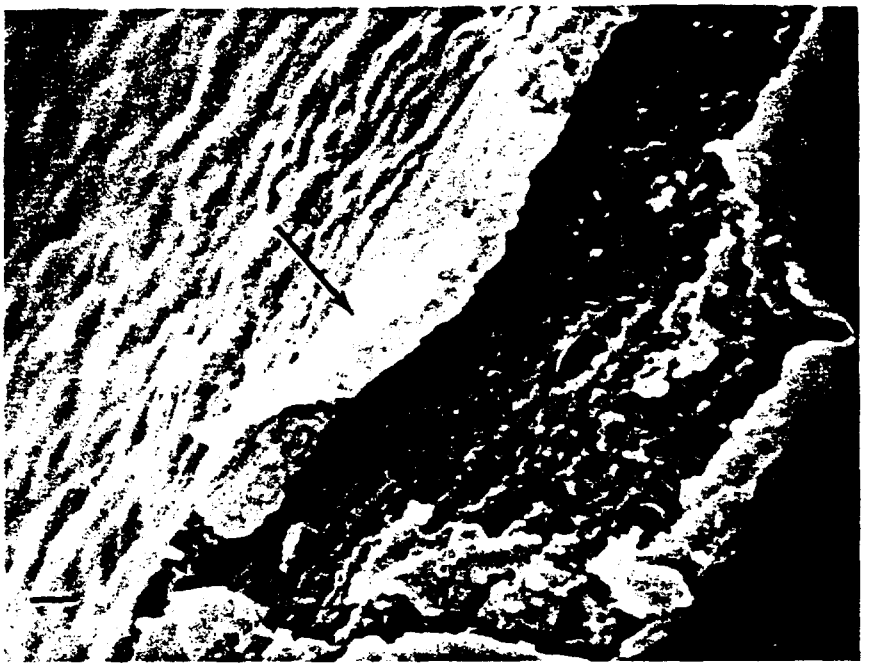


FIGURE 4. Scanning electron micrograph of a portion of the surface of a 0.2 μm Nuclepore membrane following filtration of 10 mL of untreated urine. A part of the surface mucus has been disrupted exposing the underlying membrane. The thickness of the dried mucous layer (arrow) is about 1.0 μm. ×6050. Bar = 1.0 μm.

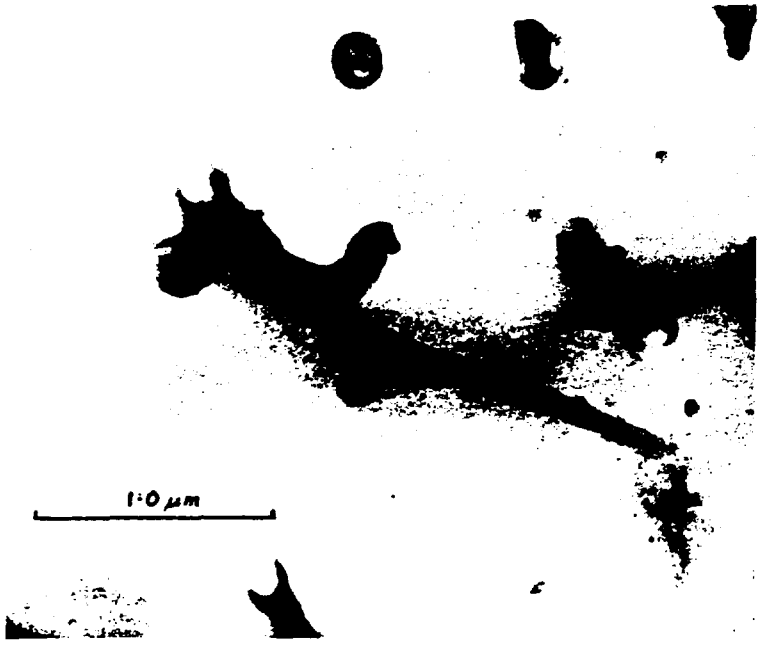


FIGURE 5. Transmission electron micrograph of a single chrysotile asbestos fiber (1.3 μm long) isolated from urine. Carbon replica of a 0.2 μm diameter pore plugged with debris is seen (arrow). ×30,000. Bar = 1.0 μm.

expect to find at least 0.4 × 10^6 fibers/L in the urine in excess of control water without consideration of the real possibility that chrysotile fibers in urine may result from exposures other than water ingestion, i.e., food, beverages, and air via lung clearance of inhaled fibers. In the second case, only 0.04 × 10^6 fibers/L would be assumed to be present, a figure well below our limit of detection.

On face value, the data would suggest that significant numbers of chrysotile asbestos fibers do not pass into the urine, whether the concentration of fibers is low or 100 times higher. With respect to these urine samples, however, additional points need to be raised: (1) How many fibers were missed due to masking by mucous residues? (perhaps about 40%). (2) How many fibers were not counted due to deterioration of their morphology and negative selected area electron diffraction patterns? (about 30%). Such corrections would obviously not raise the counts significantly. (3) To obtain a convincing difference, the average numbers of fibers in the urine samples would have to be increased by a factor of 10 or more.

The reason for not finding a significant excess of asbestos fibers in urine samples from residents of Everett may be due to a number of additional factors. First, throughout this study the attainment of a high level of detection was thwarted by the inability to filter large volumes of urines due to the presence of mucous residues in spite of pretreatment with H_2O_2. Although a level of detection of less than 0.2 × 10^6 fibers/L was obtained on urine where volumes of 50 mL or more could be filtered, the results were obscured by the generally higher background counts of the control water samples. Cook et al. (*17*) attained a similar detectable limit for amphibole fibers, and Hallenbeck et al. (*10, 11*) achieved a limit of 0.44 × 10^6 fibers/L. Both groups of workers used techniques different from ours. Second, it may be that significant numbers of chrysotile fibers do not, in fact, gain access to the excretory mechanism of the kidneys and become voided with the urine.

Cook et al. (*17*) largely avoided the mucous residue problem by ashing the Nuclepore membrane following filtration of the urine and drying. It is likely, however, as the results suggest, that a significant increase in chrysotile contamination would occur. Although Cook and Olson were looking primarily for evidence of amphibole fibers, significant numbers of contaminating chrysotile fibers that sometimes exceeded 1 × 10^6 fibers/L were found. For the past 2 yr, batches of "ashed" 47-mm diameter, 0.2-μm pore size Nuclepore membranes have consistently yielded between 6 and 21 fibers/grid opening. With this in mind, we chose to use the H_2O_2 procedure to eliminate the residual mucus from human urine. On the whole, H_2O_2 treatment appears satisfactory by light microscopy, but some mucous residues are somewhat refractory to treatment and, in addition, tend to rapidly plug the membrane pores (Fig. 5), thereby reducing filtration efficiency. A further disadvantage is that small asbestos fibers may become masked by refractory residues and escape being counted. The inability to see and to identify all fibers in a particular sample is a problem common to all asbestos analyses by TEM. Laboratory contamination by asbestos, particularly the chrysotile variety, is also a significant problem of varying magnitude. Carter and Taylor (*23*) examined 300 grid openings on 65 "blank" grids and found 1.3 fibers/grid opening.

Comparison of chrysotile fiber counts of water filtered through 0.1-μm pore size membranes and those after 0.2-μm membrane filtration yielded a fourfold increase in counts for the latter (Table 4). As yet, we have not done enough of these comparisons to be able to ascertain the implication of these results.

Cook et al. (*17*) also compared amphibole fiber counts of "spiked" urine samples after filtration and ashing with similarly treated water/amphibole suspensions without added urine and found a 29% underestimate of amphibole numbers for the urines. We recorded a 43% reduction in fiber counts (Table 4) with urine "spiked" with chrysotile asbestos and processed using H_2O_2 instead of ashing. Possibly, comparison of urine specimens between exposed and control persons is more appropriate than comparisons between urine and control water.

It is generally accepted that intralaboratory precision for asbestos counting in clean water using the interim method of Anderson and Long (*21*) is about 40-50%. This procedure also suggests that counts less than 5 fibers/20 grid openings should be considered not statistically significant. On this basis, the differences shown in Table 2 for urine largely represent counts in this range. From Tables 2 and 3, the number of fibers per liter from the Everett group of urine samples is not significantly different from the urine of the Seattle/Bellevue group. However, the control waters were significantly different due to the high counts of groups 101 to 104. In this case, it seems that there was a higher level of contamination in the early control water samples than later on. Furthermore, the fiber counts of the Everett urine samples were just significantly different (p

< 0.1, two-sided *t*-test) from their control waters. This is most likely due to an unknown loss of fibers by masking by residues of debris. A 43% loss of fibers assumed, as judged by the "spiked" urine counts, is consistent with the difference between Everett urine samples and control water.

At this point, some functional and structural aspects of the kidneys should be reviewed. The output of urine in normal adult subjects varies from 600 to 2500 mL/day. The blood flow through the combined kidneys is 1200 mL/min. Each kidney has about 1×10^6 nephrons, and each nephron consists of a glomerulus (filtration unit) connected to a long tubule consisting of four parts: (1) proximal convoluted tubule, 60 μm diameter (resorption); (2) Loop of Henle; (3) distal convoluted tubule, 20–50 μm diameter; and (4) collecting tubule 100–200, μm diameter. From this last tubule follows the renal pelvises, ureter, and, finally, the bladder. It is the mucous coats of the ureters and bladder that give rise to mucus in the urine. Not to be forgotten is the generous system of lymphatics, which serves the kidneys, ureters, and bladder. A glomerular filtration unit (Fig. 6) consists of capillary endothelium punctuated by regularly spaced pores, 50–100 μm in diameter; fused basement membranes (BM) of the overlying podocyte foot processes and underlying endothe-

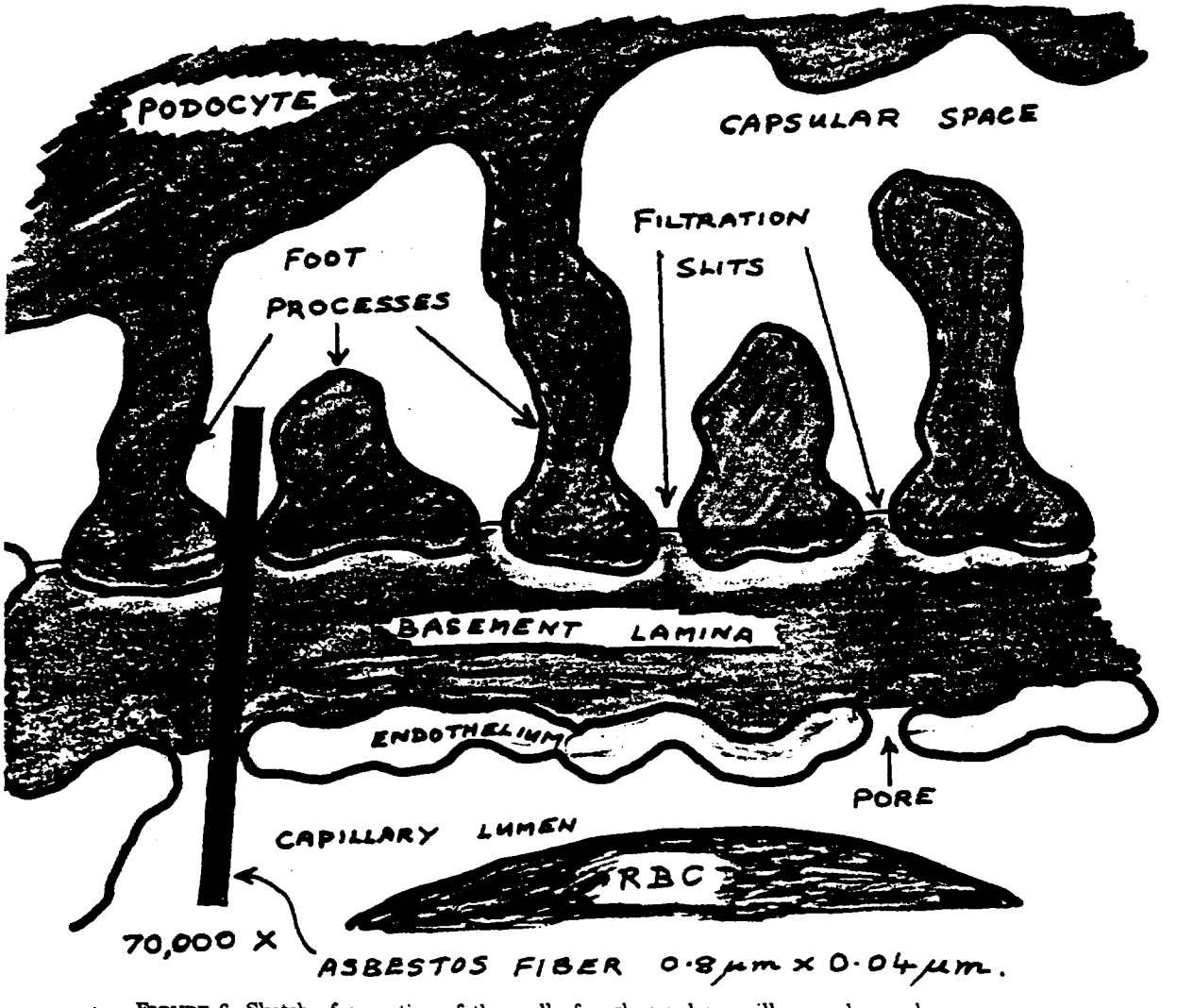


FIGURE 6. Sketch of a portion of the wall of a glomerular capillary and capsular space. Modified from Bloom and Fawcett (*24*). A hypothetical chrysotile asbestos fiber (0.8 μm × 0.04 μm) has been drawn in as a size reference. ×70,000.

lial cell form a homogenous barrier, which is believed to be the principal filter to hold back large molecules in conjunction with filtration slits formed between any two adjacent foot processes.

Two potential sources of asbestos fiber contamination of renal tissue are therefore the blood plasma and the lymph, and two potential pathways for access into the urine are (1) entry through the endothelial pore–basement membrane–filtration slit network, and (2) into the bladder via the lymphatics. That particulate matter can be excreted along with the urine is suggested by the works of Cook and Olson (*17*) with waterborne amphiboles, Bignon et al. (*13*) with orally ingested attapulgite clay, and Hallenbeck et al. (*10*) with ingested chrysotile and crocidolite. At present, the question of how fibers find their way into the urine is unresolved. One might ask if fiber surface charge has anything to do with the process of entry or if it is simply a spearlike penetration by a relatively rigid rod-shaped object. An equally important aspect to consider is not the numbers of fibers retrieved from urine—thus safely and permanently removed—but how many fibers are sequestered in kidney tissue. The latter is an even more difficult problem to solve given the ubiquitous nature of contaminating chrysotile.

It is clear that there are substantial difficulties associated with the estimation of chrysotile asbestos fibers in human urine by the use of present techniques. The difficulties arise from the inherent chrysotile contamination of the 0.2-μm Nuclepore filters, the presence of residual mucous material on the membrane, and, finally, sporadic environmental contamination of the sample during processing. At the present time, the results of the present study can only be regarded as inconclusive but would suggest no relationship between high numbers of chrysotile fibers in drinking water and the numbers estimated for voided urine. For whatever reason, no urine examined showed high numbers of chrysotile fibers during the 21 months of intermittent observation. The resolution of this problem will rest on future procedures to rid urine of obstructive mucous, on the acquisition of membrane filters free of endogenous chrysotile asbestos, and on obtaining a larger group of urine donors, which, to detect a significant difference between the mean test value and the mean control value, would require a considerable increase over the present numbers and consideration given to the frequency of sampling.

The authors thank Dr. Claris Hyatt, Health Officer, Snohomish Health District, and her staff for their splendid cooperation during this study, and Roy Jones, Physical Scientist, EPA, Region X, Seattle, for his continued interest in this work from its conception. This Research was supported by Contract No. CI790578 from the U.S. Environmental Protection Agency.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Cunningham, H. M., and Pontefract, R. Asbestos fibers in beverages and drinking water. Nature 232:332–33? (1971).
2. Cook, P. M., Glass, G. E., and Tucker, J. H. Asbestiform amphibole minerals: detection and measurement of high concentrations in municipal water supplies. Science 185: 853–855 (1974).
3. Millette, J. R., Clark, P.J., and Pansing, M. F. Exposure to Asbestos from Drinking Water in the United States. Environmental Health Effects Research Report, Office of Research and Development, EPA-600/1-79-028, 1979.
4. Millette, J. R., Boone, R., and Rosenthal, M. Asbestos in Cistern Water. Environmental Health Effects Research Report, Office of Research and Development. EPA-657-093-7105, 1980.
5. Chatfield, E. J., and Dillon, M. J. A national survey for asbestos fibres in Canadian drinking water supplies. Environmental Health Directorate, Ottawa, 79-EHD-34, 1974.
6. Gaudichet, A., Bientz, M., Sebastien, P., Dufour, G., Bignon, J., Bonnaud, G., and Puissais, J. Asbestos fibers in wines: relation to filtration process. J. Toxicol. Environ. Health 4: 853–860 (1978).
7. Biles, B., and Emerson, T. R. Examination of fibres in beer. Nature 219: 93–94 (1968).
8. Cunningham, H. M., Pontefract, R. D., and O'Brien, R. Quantitative relationship of fecal asbestos to asbestos exposure. J. Toxicol. Environ. Health 1: 377–379 (1976).
9. Cunningham, H. M., Moodie, C. A., Lawrence, G. A., and Pontefract, R. D. Chronic effects of ingested asbestos in rats. Arch. Environ. Contam. Toxicol. 6: 507–513 (1977).
10. Hallenbeck, W. H., and Patel-Mandlik, K. J. Presence of fibers in the urine of a baboon gavaged with chrysotile asbestos. Environ. Res. 20: 335–340 (1979).
11. Hallenbeck, W. H., Markey, D. R., and Dolan, D. G. Analyses of tissue, blood and urine samples from a baboon gavaged with chrysotile and crocidolite asbestos. Environ. Res. 25: 349–360 (1981).
12. Sebastien, P., Masse, R., and Bignon, J. Recovery of ingested asbestos fibers from the gastrointestinal lymph in rats. Environ. Res. 22: 201–216 (1980).
13. Bignon, J., Sebastien, P., Gaudichet, A., and Jaurand, M. C. Biological effects of attapulgite. In: Biological Effects of Mineral Fibres, Vol. 1 (J. C. Wagner, Ed.) IARC, Lyon, France, 1980, pp. 163–181.
14. Kanarek, M. S., Conforti, P. M., Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer incidence in the San Francisco Bay area. Am. J. Epidemiol. 112: 54–72 (1980).
15. Conforti, P. M., Kanarek, M. S., Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer in the San Francisco Bay area: 1969–1974 incidence. J. Chron. Dis. 34: 211–224 (1981).
16. Polissar, L., Severson, R. K., Boatman, E. S., and Thomas

D. B. Cancer incidence in relation to asbestos in drinking water in the Puget Sound Region. Am. J. Epidemiol. 116: 314–328 (1982).

17. Cook, P. M., and Olson, G. F. Ingested mineral fibers: elimination in human urine. Science 204: 195–198 (1979).
18. Levy, B. S., Sigurdson, E., Mandel, J., Laudon, E., and Pearson, J. Investigating possible effects of asbestos in city water: surveillance of gastrointestinal cancer incidence in Duluth, Minnesota. Am. J. Epidemiol. 103: 362–368 (1976).
19. Sigurdson, E. E., Levy, B. S., Mandel, J., McHugh, R., Michienzi, L. J., Jagger, H., and Pearson, J. Cancer morbidity investigations: lessons from the Duluth study of possible effects of asbestos in drinking water. Environ. Res. 25: 50–61 (1981).
20. Chatfield, E. J., Glass, R. W., and Dillon, M. J. Preparation of Water Samples for Asbestos Fiber Counting by Electron Microscopy. Environmental Research Laboratory Report, Office of Research and Development, Cincinnati. EPA-600/4-78-011, 1978.
21. Anderson, C. H., and Long, J. M. Interim Method for Determining Asbestos in Water. Environmental Research Laboratory Report, Office of Research and Development, Athens, GA, EPA-600/4-80-005, 1980.
22. Millette, J., and Rosenthal, M. Fate of ingested particulates. In: Proceedings, National Workshop on Substitutes for Asbestos. EPA Report 560/3-80-001, Cincinnati, 1980, pp. 313–327.
23. Carter, R. E., and Taylor, W. F. Identification of a Particular Amphibole Asbestos Fiber in Tissues of Persons Exposed to a High Oral Intake of the Mineral. Report, Environmental Health Advisory Panel, American Public Health Association, Washington, D.C., 1978.
24. Bloom, W., and Fawcett, D. W. Textbook of Histology, 9th ed., Saunders, Philadelphia, 1968.

Environmental Health Perspectives
Vol. 53, pp. 143–148, 1983

How Are The Physical and Chemical Properties of Chrysotile Asbestos Altered by a 10-Year Residence in Water And up to 5 Days in Simulated Stomach Acid?

by Krisna Seshan*

Although there have been a number of studies on the ingestion of asbestos, few studies exist on how the chrysotile asbestos itself is altered by the exposure to the acid stomach environment. This study has found that there are changes in the physical, chemical and surface properties of chrysotile asbestos as a result of exposure to water, strong acids, and simulated gastric juices. It was observed that the charge on the surface (the zeta potential) is changed from positive to negative; the surface becomes silicalike; and the magnesium is lost from the fibers of asbestos upon exposure to water and acid. It was also noted that the smaller the fiber diameter, the faster the loss of the magnesium.

Notable among the changes in physical properties is a change in the refractive index. This means that asbestos exposed to acids or water may not be detectable using the dispersion staining techniques that identify asbestos based on the refractive index. Other physical property changes include the destruction of the gross crystallinity of the fibers. The x-ray diffraction signal disappears when fibers are exposed to acid. However, this study shows that the fibers may still be detected by electron diffraction.

It appears that upon acid exposure, the magnesium ions are leached out, leaving a magnesium-free silica network. A positive ion, possibly the proton (H^+) or the hydronium ion (H_3O^+), replaces the lost magnesium ion.

Introduction

The prevalence of asbestos in drinking water in the United States has been cataloged (*1,2*), and the effects of magnesium leaching on the biological effects of inhaled asbestos have been studied (*3–5*). However, no studies have been found that have considered the effects of magnesium leaching in ingested asbestos.

Several mineralogists (*6,7*) have studied how asbestos changes under heat and presssure, while others (*8,9*) have studied the surface properties of asbestos fibers. The physical and chemical properties of asbestos have been cataloged (*10,11*). The optical properties of chrysotile have been studied (*12*), and dye absorption on chrysotile has been investigated (*13,14*). The study summarized here attempted to use these various techniques and tests on asbestos fibers altered by exposure to simulated gastric juices and fibers stored in water for long periods of time. Specific details of the studies can be found in the more comprehensive final report, which will be available from the U.S. Environmental Protection Agency (U.S. EPA) at a future date.

This work impacts two areas. The first is the area of chrysotile identification in the environmental matrix. Acid-treated fibers may not be easily detectable by conventional techniques used for asbestos identification. Second, there is the biomedical implication: What changes in fiber properties caused by exposure to water or gastric juice will alter the biological effects of asbestos? Light and Wei have pointed out the possibility of a connection between surface charge and toxicity (*15*).

*Department of Metallurgical Engineering, University of Arizona, Tucson, AZ 85721.

Materials and Methods

This project studied the changes in the physical, chemical, and surface properties of chrysotile asbestos after exposure to doubly distilled (DD) water for 10 years, 1 N hydrochloric acid for 1/2-hr to 5-day intervals, and to simulated gastric juices for up to 5 days. The simulated gastric juices were produced by adding 2 g of NaCl, 3.2 g of pepsin (hog extract), and 7.0 mL of HCl to a liter of distilled water (*16*). The pH of the juice was 1.2.

Chrysotile asbestos samples were obtained from three sources: International Union Against Cancer (UICC), National Institute of Environmental Health Sciences (NIEHS), and Globe, AZ.

A variety of tests, including X-ray and electron diffraction, energy-dispersive X-ray analysis, and various surface tests, were performed. The tests may be divided into three main categories: physical, chemical, and surface charge investigations.

Changes in surface charge as a result of acid exposure were determined by the measurement of zeta potential (ZP) (*17,18*) versus pH. Untreated fibers were compared with those treated in 1 N HCl and those treated in simulated gastric juices. A nitrogen absorption (*19*) experiment was performed to determine changes in surface area. Changes in surface were also studied by a dye adsorption method (*20*).

Physical changes to the fiber after acid exposure were found by measuring the refractive index of the fibers. X-ray and electron diffraction were used to study the differences between acid-treated, water-treated, and untreated fibers.

Chemical changes to the fiber after acid treatment were studied by performing atomic absorption (AA) analyses of the liquid in which the fibers were treated. The total amount of magnesium lost from the fibers was determined. Energy-dispersive X-ray analysis on individual fibers was performed using a scanning transmission electron microscope (STEM). In this case the Mg/Si ratio, as a function of residence time in acid, was determined.

Results

Upon exposure to simulated gastric juice, the ZP of NIEHS, UICC, and Globe chrysotile asbestos goes from positive to negative in less than 1 hr (Fig.1). Figure 2 shows the results of asbestos exposure to 1 N HCl. In 8 hr the ZP still remained positive. The different results suggest that the NaCl and pepsin have an important part to play in changing surface charge.

ZP-pH measurements can be used to understand surface changes. Figures 3 and 4 compare ZP-pH curves for untreated and 0.1 N HCl-treated chrysotile. The point at which the ZP curve cuts the x-axis, called the zero point charge (ZPC), has moved from a pH of 6.5 for untreated fibers to a pH of 4 for the treated chrysotile. Since chrysotile has a ZPC of 6 and silica has a ZPC of 4, Figures 3 and 4 show that acid exposure has turned the chrysotile surface to a silicalike surface.

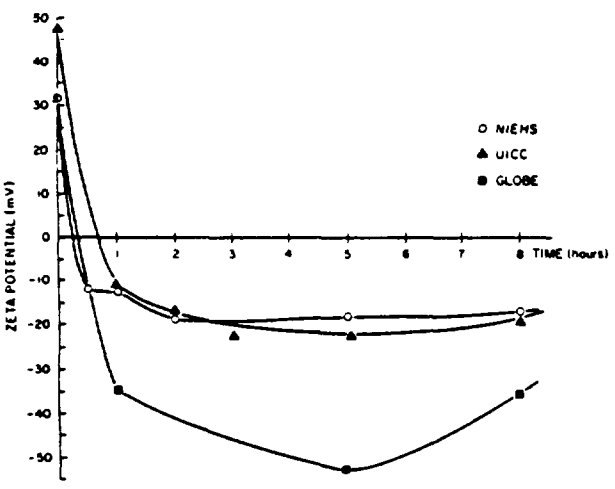


FIGURE 1. Plot showing the variation of zeta potential of chrysotile asbestos fibers during an 8-hr treatment in simulated gastric juice.

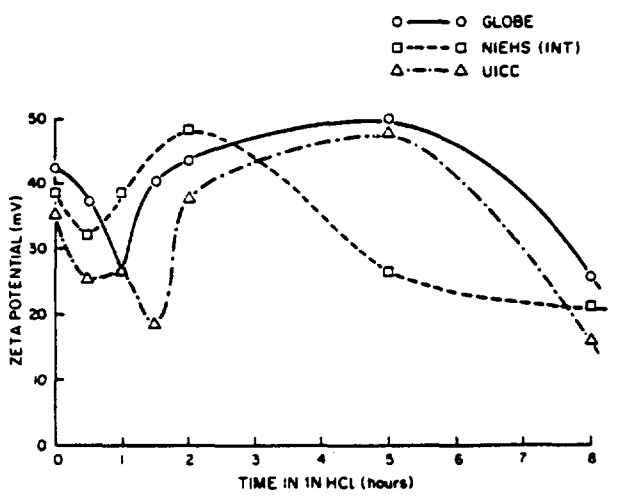


FIGURE 2. Plot showing the variation of zeta potential of chrysotile asbestos from several sources during an 8-hr treatment in 1 N HCl.

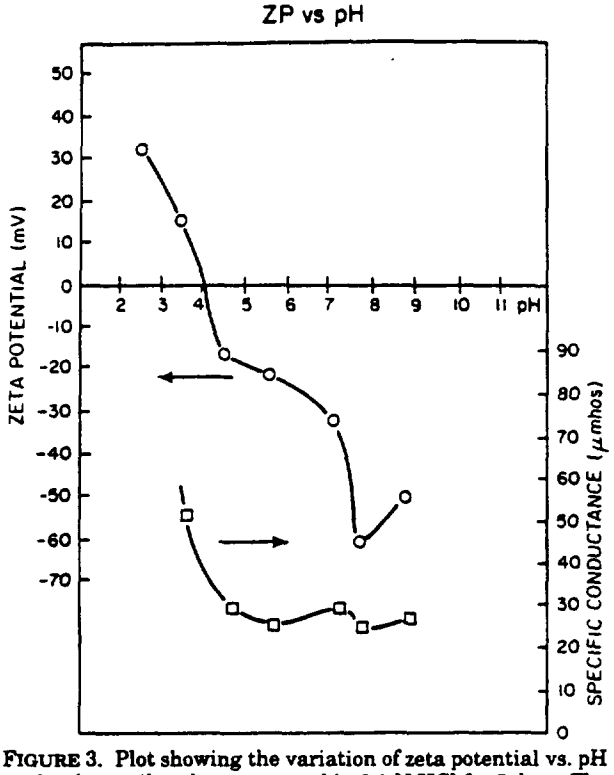


FIGURE 3. Plot showing the variation of zeta potential vs. pH for chrysotile asbestos treated in 0.1 N HCl for 5 days. The specific conductance of the solution is shown at the bottom, for calibration purposes.

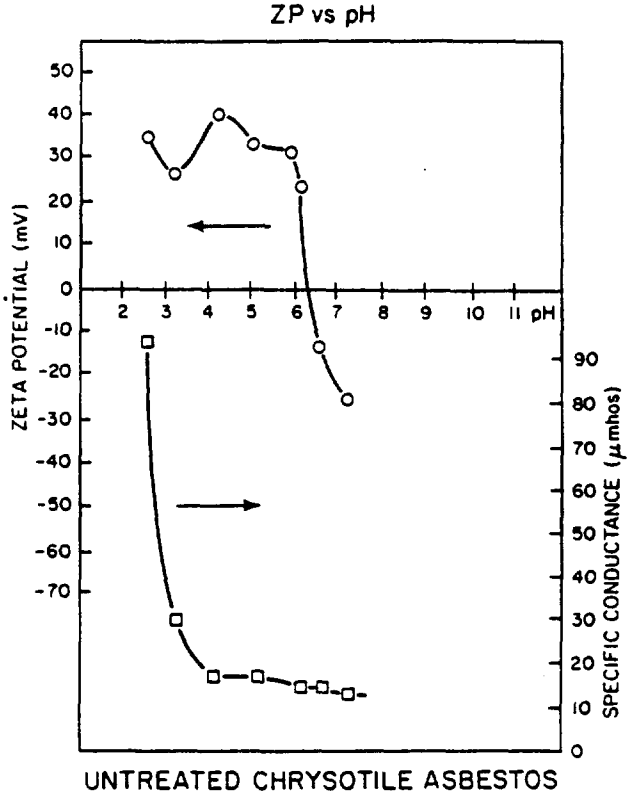


FIGURE 4. Plot showing the variation of zeta potential vs. pH for chrysotile that has not been treated.

Table 1. Surface area of chrysotile asbestos determined using the nitrogen adsorption technique.

Sample	Treatment	Surface area, m^2/g
Globe (fine)	Untreated	29.55
NIEHS (intermediate)	Untreated	29.76
Globe (fine)	2 hr, 1 N HCl	30.99
NIEHS (intermediate)	2 hr, 1 N HCl	59.82
Globe (fine)	Sonicated	21.21
NIEHS (intermediate)	Sonicated	21.50

Table 1 shows that the surface area of the treated NIEHS chrysotile is about double that of the untreated chrysotile as measured by the nitrogen adsorption experiment. The chrysotile from Globe was not affected by the acid treatment. The difference cannot be attributed to larger fibers in the NIEHS material breaking up into smaller fibers with more surface area because there was no difference in nitrogen adsorption after sonication of both materials.

The results of the dye adsorption measurement of surface area are shown in Figure 5. The HCl is shown to have the greatest effect on the asbestos fibers.

One of the important physical properties that changes upon acid treatment of chrysotile is its refractive index. This change for 1 N HCl and simulated gastric juice treatments is shown in Figure 6. The refractive index decreases from 1.54 for the untreated fiber to 1.44 for the treated fibers. A refractive index of 1.4 is approaching that of the zeolite minerals. This is consistent with the removal of magnesium from the chrysotile, leaving an open framework type of silicate. The result suggests that methods used to identify chrysotile based on its refractive index alone will not be effective in identifying acid-treated fibers.

Acid treatment also destroys the X-ray diffraction pattern of chrysotile. The effect on the X-ray pattern of chrysotile after 3 and 5 days in 1 N HCl is shown in Figure 7. Crocidolite, an amphibole asbestos form, remains unchanged (Fig. 8). The results of an electron diffraction study of acid-treated chrysotile are shown in Table 2. It was observed that after 5 days in 1 N HCl, the electron diffraction patterns lost clarity; this is assumed to be related to the loss of magnesium ions from the fiber.

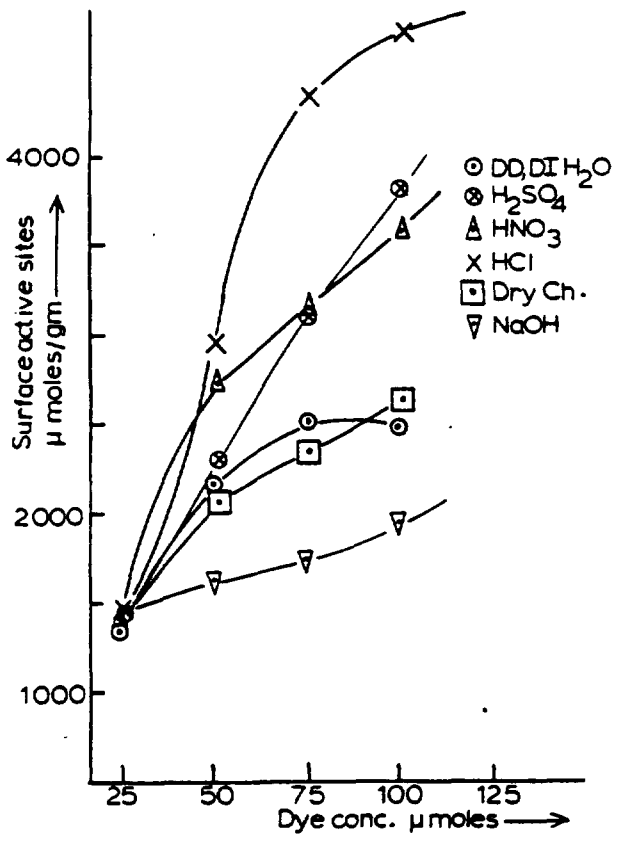


FIGURE 5. Results of surface area measurement using a dye adsorption technique. Chrysotile asbestos from Globe, AZ.

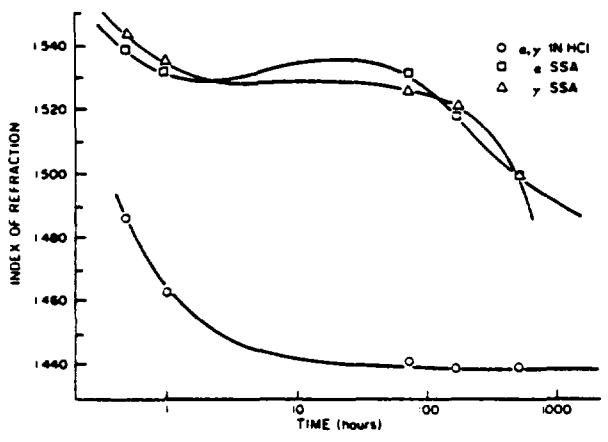


FIGURE 6. Graph showing the refractive index of Globe chrysotile vs. the time treated in 1 N HCl and in simulated gastric juice. α is the refractive index perpendicular to the fiber axis. γ is the index along the fiber axis. Treatment in HCl cause α and γ to become equal and drop to a value of 1.44.

Table 2. Chrysotile fibers identifiable by electron diffraction.[a]

	Fibers identifiable, %		
Source	Untreated	30 min, 1 N HCl	5 days, 1 N HCl
UICC	89	67	60
NIEHS	90	80	77
Globe, AZ	92	87	80

[a]At least three layer lines must be visible to be considered identifiable for this test.

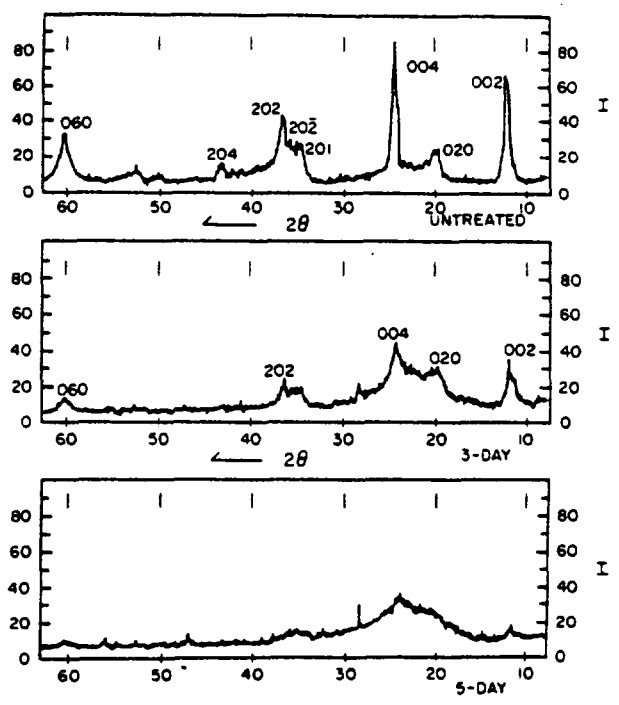


FIGURE 7. X-ray diffractometer trace of intensity vs. 2 θ for 1 N HCl-treated chrysotile asbestos.

Chrysotile asbestos has the formula $Mg_3[Si_2O_5](OH)_4$. The results of the AA analysis of the liquid in which the asbestos materials had been placed (Fig.9) leads to the clear conclusion that acid leaches magnesium from chrysotile fibers. The excellent agreement with electron microprobe results of Monchaux et al. (4) may also be seen in Figure 9.

The X-ray microanalysis results shown in Figures 10 and 11 show that the smaller the fiber, the greater the magnesium loss. This implies that the loss of magnesium is from the surface of the fiber rather than from the ends.

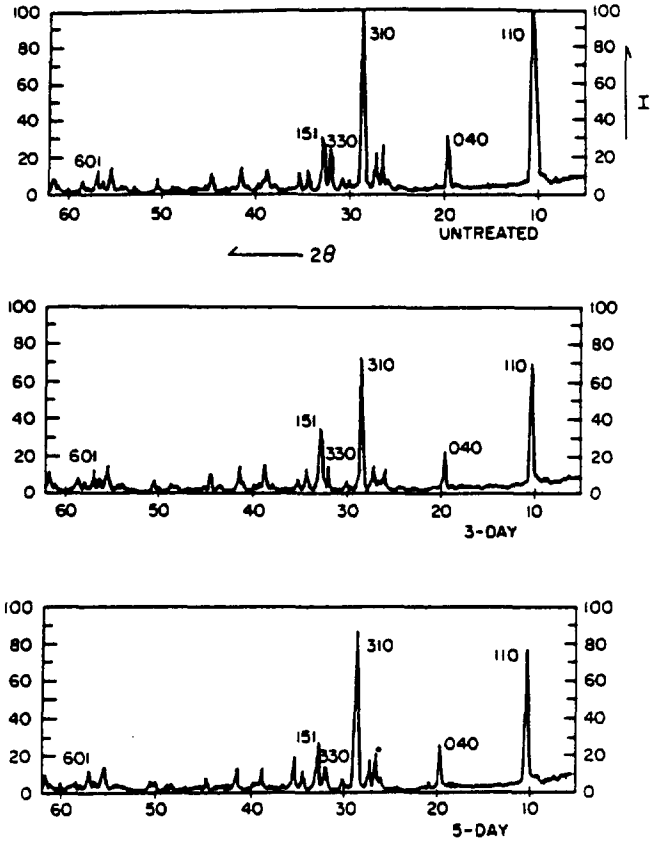


FIGURE 8. X-ray diffractometer trace of intensity vs. 2 θ for 1 N HCl-treated crocidolite.

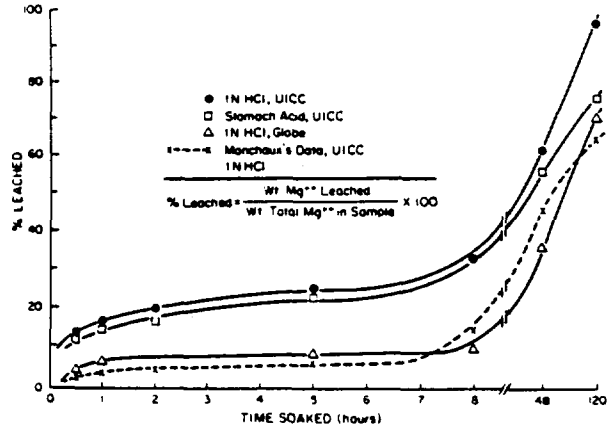


FIGURE 9. Percent magnesium as function of time. Comparison with the results of Monchaux et al. (4).

Discussion

Some of the changes caused by HCl and gastric acid would make it difficult to identify acid-treated fibers. It will be necessary to develop new

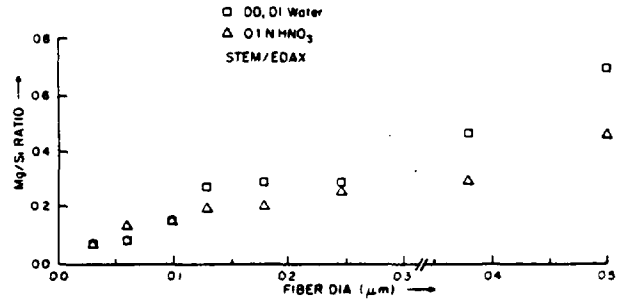


FIGURE 10. Magnesium to silicon ratio vs. fiber diameter for Globe chrysotile in water and in 0.1 N HCl.

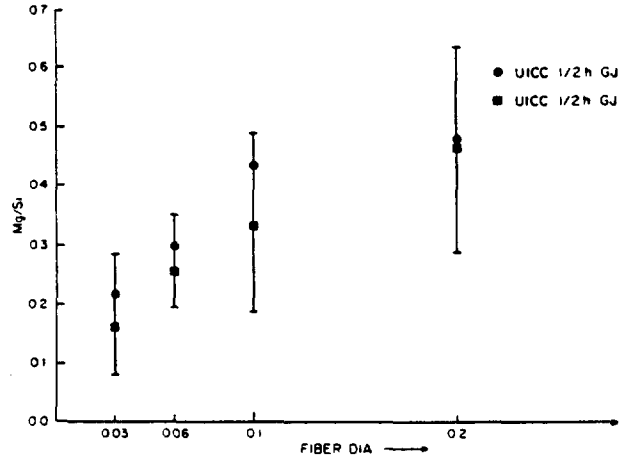


FIGURE 11. Magnesium to silicon ratio vs. fiber diameter for UICC chrysotile treated with simulated gastric juice.

techniques for the optimum identification of fibers after they have been placed in the gstrointestinal tract through ingestion.

The simulated gastric juices used in this study did not contain all components of human stomach acid. The complex organic compounds such as muco- and glycoproteins may play a large part in coating the fibers after they are in the stomach and have a great effect on such parameters as surface charge and magnesium leaching.

Conclusions

Chrysotile asbestos from three sources (UICC, NIEHS, and Globe, Arizona) has been shown to change its physical, chemical and surface properties after exposure to HCl and simulated gastric juice.

The research described in this report was supported by Grant No. CR80700 from the U.S. Environmental Protection Agency and Grant No. BRSG S07 RR07002 awarded by the Biomedical Research Support Grant Program, Division of

Research Resources, International Institutes of Health. The assistance of J. Millette and that of my various students is gratefully acknowledged.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Millette, J. R., and McFarren, E. F. EDS of waterborne asbestos fibers in TEM, SEM, and STEM. SEM/1976 9: 451–460 (1976).
2. Millette, J. R., Clark, P. J., Pansing, M. F., and Twyman, J. D. Concentration and size of asbestos in water supplies. Environ. Health Perspect. 34: 13–24 (1980).
3. Wagner, J. C., Berry, G., and Timbrell, V. Mesotheliomas in rats after inocculation with asbestos and other materials. Brit. J. Cancer 28: 173–185 (1973).
4. Monchaux, G., Bignon, J., Jaurand, M. C., Lafuna, J., Sebastian, P., Masse, R., Hirsch, A., and Goni, J., Mesotheliomas in rats following inoculation with acid leached chrysotile asbestos and other mineral fibers. Carcinogenesis 2: 229–236 (1981).
5. Morgan, A., Davies, P., Wagner, J. C., Berry, G., nad Holmes, A. The biological effect of magnesium leached chrysotile asbestos. Brit. J. Exptl. Pathol. 58: 465–473 (1977).
6. Chen, Y. Y. T. IR studies of acid, bases, heat and pressure on asbestos and structurally related substances. J. Assoc. Anal. Chem. 60: 1266–1278 (1977).
7. Choi, I., and Smith, R. W. Kinetic study of the dissolution of asbestos fibers in water. J. Colloid. Interface Sci. 40: 253–262 (1971).
8. Fripiat, J. J., and Faille, M. D. Surface properties and texture of chrysotile. Clays Clay Min. 15: 305–320 (1966).
9. Holt, P. F., and Clark, S. G. Dissolution of chrysotile asbestos in water, acid, and alkalies. Nature 185: 237–253 (1966).
10. Papirer, E., and Roland, P. Grinding of chrysotile in hydrocarbons, alcohol, and water. Clays Clay Min. 29: 161–175 (1981).
11. Pundsak. F. L. The properties of asbestos. I. J. Phys. Chem. 59: 892–895 (1955).
12. Bloss, F. D. An Introduction to the Methods of Optical Chrystallography. Holt, Rinehart, and Winston, New York, 1961, p. 59.
13. Gurr, E. Synthetic Dyes in Biology, Medicine and Chemistry. Academic Press, London, 1971, pp. 288–291.
14. Markham. M. C., and Wosczyna, K. Determination of microquantities of chrysotile asbestos by dye adsorption. Environ. Sci. Technol. 10: 930–931 (1976).
15. Light, W. G., and Wei, E. T. Surface charge and hemolytic activity of asbestos. Environ. Res. 13: 135–145 (1977).
16. U. S. Pharmacopeia. Rockville, MD, 1979, p. 1105.
17. Chowhury, S., and Kitchner, J. A. Zeta potentials of natural and synthetic chrysotiles. Intern. J. Min. Proc. 2: 277–285 (1975).
18. Ralston, J., and Kitchner, J. A. The surface chemistry of amosite asbestos, an amphibole silicate. J. Colloid Interface Sci. 50: 242–249 (1975).
19. Naumann, A. W., and Dresher, W. H. Colloidal suspensions of chrysotile asbestos: specific ion effects. J. Phys. Chem. 70: 288–291 (1966).
20. Rose, H. A. Detection and determination of chrysotile in talc USP. J. Pharm. Sci. 63: 268-269 (1974).

Environmental Health Perspectives
Vol. 53, pp. 149–152, 1983

Transmigration of Ingested Asbestos

by M. E. Meek*

There has been speculation that the ingestion of asbestos in food and drinking water may play some role in the etiology of cancer of the gastrointestinal (GI) tract. An important question in the evaluation of the possible human cancer risk associated with asbestos ingestion is whether fibers can penetrate into and through the GI tract in sufficient numbers to cause adverse systemic or local effects.

Factors that complicate interpretation of the available data on the transmigration of ingested asbestos are discussed, and the preliminary results of our ongoing investigation of the penetration of amosite fibers into the normal and abnormal intestinal mucosa of the Wistar rat are reported.

Introduction

An important question in the evaluation of the possible human cancer risk associated with the ingestion of asbestos is whether fibers can migrate from the lumen into and through the walls of the gastrointestinal tract in sufficient numbers to cause adverse systemic or local effects. There is considerable disagreement concerning this subject.

There has been evidence of transmigration in studies involving electron microscopic examination of tissue residues or urine of humans in which exposure was assumed to be via the gastrointestinal route (*1,2*) or in animals following introduction of asbestos into the lumen of the GI tract (*3–8*). On the other hand, there has been no evidence of penetration in one study involving electron microscopic examination of the fiber content of human urine (*9*) and in several studies in which tissue residues were examined by electron microscopy following oral administration of asbestos to various species of animals for various periods of time (*10–13*).

Moreover, in those studies with positive results, there has been conflicting evidence concerning the dimensions of fibers which transmigrate, with Sebastien et al. (*8*) reporting preferential passage for long fibers and Cook and Olson (*2*) reporting greater transmigration of shorter fibers. The available data are inconclusive due to several factors which complicate the interpretation and comparison of the results of the studies involving electron microscopic examination of the fiber content of tissue residues or biological fluids. In several of the studies in which there was no evidence of widespread transmigration, the sensitivity of the analytical technique was not well characterized and it is possible that the method was not sufficiently sensitive (*10,11*). Alternatively, fibers may have been lost during sample preparation.

It is also extremely difficult to avoid contaminating samples from external sources. In some cases, there may be contamination that is not monitored in blank sample preparation and analysis. For example, in a study conducted by Carter and Taylor (*1*) amphibole fibers were identified in ashed tissue samples of residents of Duluth (where concentrations of up to 100×10^6 fibers/L have been measured in the drinking water supply). However, it is possible that the tissue samples of the Duluth residents may have been contaminated since it has been previously reported that the formalin used to fix autopsy specimens in some Duluth hospitals was diluted with city tap water containing amphibole asbestos fibers (*14*). There is also the possibility of cross contamination from the gut lumen in the animal studies designed to examine fiber content of tissue residues following oral administration of asbestos, particularly since asbestos fibers adhere firmly to the gut surface; scanning electron microscopy has revealed the presence of fibers on the surface of the gastrointestinal tract, even after vigorous

*Monitoring and Criteria Division, Bureau of Chemical Hazards, Environmental Health Directorate, Department of National Health and Welfare, Ottawa, Canada KIA 0L2.

washing of the mucosa at autopsy (*11*). In some of the studies, there was insufficient time between feeding of the asbestos and killing of the animals to allow clearance of fibers from the gastrointestinal tract (*7*). In addition, at autopsy, it is difficult to avoid contamination of tissues by fibers which may have adhered to the fur of the animal during feeding. As a result, the fiber content of tissues in the asbestos-fed animals may be higher than that in the control animals, due to contamination. In several of the studies reporting positive results, no attempt was made to replicate fiber counts, and there were relatively few control subjects or animals (*2,8*).

There have also been several studies in which thin samples of the gastrointestinal mucosa, rather than bulk tissue residues, have been examined for fiber content by electron microscopy following introduction of asbestos into the lumen of the GI tract of animals (*10,15–17*). The results of these studies have also been contradictory; fibers have been identified in epithelial cells and the lamina propria in some but not all of these investigations. However, only small areas of tissue can be examined in this manner and it has been suggested that fibers may be forced into (*11,16*) or dragged from the cells during thin section preparation. Similarly, the results of studies concerning the effects of ingested asbestos on proliferation and other biochemical parameters in epithelial cells of the gastrointestinal tract have also been contradictory (*11,18–21*).

Although fibers have been identified in tissue residues or biological fluids of animals and humans ingesting asbestos, there has been no conclusive confirmatory evidence of a tissue response associated with penetration of the gastrointestinal epithelium. Jacobs et al. (*17*) observed light and electron microscopic evidence of cellular damage in the intestinal mucosa of rats fed 0.5 or 50 mg of chrysotile per day for 1 week or 14 months. However, most of the changes were nonspecific and are commonly observed in the mucosae of control animals. Such changes may have occurred in the absence of actual fiber penetration; in fact, no fibers were detected in the mucosa of the treated animals upon examination by electron microscopy. The presence of "iron-containing" macrophages in the duodenal and ileal mucosae of baboons fed asbestos for up to 5 years has been reported; however, few data were presented concerning the study protocol (*22*). Confirmation of such a pathological response would help to verify that fibers in the gastrointestinal wall or other tissues in previous studies were not present due to contamination from the gut lumen or from exogenous sources which may have occurred during tissue processing.

Experimental Investigation and Results

We have, therefore, been conducting animal studies to investigate the tissue response associated with the presence of asbestos fibers in the wall of the intact gastrointestinal tract. In our first experiment, histopathological sections of duodenal tissues of two 200 g Wistar rats were examined by light microscopy 4 days following the injection of 0.1 mL suspensions of UICC amosite fibers in physiological saline into the wall of the duodenum during laparotomy. A 10 mL volume of the upper layer of a solution of 3.9 μg of UICC amosite in 20 mL of physiological saline was used as the stock solution for the injections. This is an nonphysiological route of administration and does not simulate the exposure of man to ingested asbestos. However, this experiment was conducted solely to investigate and characterize the short-term tissue response associated with the presence of amosite in the wall of the gastrointestinal tract.

Granulomas which were characterized by dense masses of macrophages were present at the sites of injection of amosite; intracellular crystals with the polarizing characteristics of amosite were clearly visible. A smaller and more localized tissue reaction was evident at the sites of injection of physiological saline in two control animals. These granulomas were characterized by few capillaries and fibroblasts and relatively scanty macrophages.

Having characterized the tissue response associated with the presence of amosite in the wall of the gastrointestinal tract, intestinal tissues of Wistar rats were examined by light microscopy for pathological changes following the ingestion of asbestos. Although there may be gastrointestinal-induced changes in ingested fibers, it seems likely that penetration of amosite into the gut wall would provoke a macrophage response which would result in infiltration of macrophages, since asbestos fibers induce a well-marked granulomatous condition in the lungs and since a similar response was evident in the wall of the gastrointestinal tract several days after the injection of amosite in this study. However, there was no evidence of a macrophage response or other pathological changes in the small intestine of animals 5 days following administration of 100 mg UICC amosite by gavage daily for 5 days.

Therefore, there was no pathological evidence of penetration of amosite asbestos into the gastrointestinal mucosa of Wistar rats, based on examination by light microscopy. Although the method employed in this investigation does not preclude entirely the possibility of limited penetration of small fibers, these results are consistent with the lack of widespread transmigration reported in several studies involving electron microscopic examination of tissue residues following oral administration of asbestos to various species of animals for various periods of time (*10–12*).

We have also examined in a preliminary fashion, the extent of transmigration in regions of ulceration. This is an area of considerable interest, particularly in light of the fact that it is estimated that up to 10% of all males between the ages of 20 and 50 have areas of intestinal mucosal damage (*23*). A 100 mg portion of UICC amosite suspended in corn oil was administered by gavage to six female Wistar rats daily for 2 days; six control rats received a similar volume of corn oil at the same time. On the third day, 10 mg/kg indomethacin was administered by gavage to both asbestos-treated and control animals to induce ulcers. The asbestos-treated animals received a further 25 mg UICC amosite on the same day and daily thereafter until they were killed sequentially at intervals of approximately 24, 48, 54, 72, 96 and 120 hr following indomethacin administration. The indomethacin-control group received similar volumes of corn oil and were killed on the same schedule. At postmortem, areas of the gastrointestinal tract with visible signs of ulceration were removed and processed for histological examination by light microscopy. No intracellular fibers were observed upon polarizing light microscopic examination of the areas of ulceration.

Conclusions

These preliminary results indicate that the gut wall of rats may present an effective barrier to the penetration of asbestos even under conditions of loss of the epithelium. However, there are several limitations inherent in this investigation and additional studies are underway. For example, the administration period may not have been sufficiently long or further study involving electron microscopic examination of macrophages in the vicinity of gastrointestinal ulcers might reveal the presence of fibers of sizes which are below the limit of resolution of the light microscope. In our further studies, mucosal cells of the intact GI tract will be examined by electron microscopy for pathological evidence of fiber penetration following prolonged oral administration of asbestos in combination with a low-fiber diet. Similarly, macrophages in the vicinity of gastrointestinal ulcers in both acute and chronic stages will be examined for the presence of fibers by electron microscopy. This will be combined with an experiment to determine the likelihood of extraction of fibers during preparation of thin tissue sections.

The work described in this paper was not funded by the U.S. Environmental Protection Agency. The contents do not necessarily reflect the view of the Agency and no official endorsement should be inferred.

REFERENCES

1. Carter, R. E., and Taylor, W. F. Identification of a particular amphibole asbestos fiber in tissues of persons exposed to a high oral intake of the mineral. Environ. Res. 21: 85–93 (1980).
2. Cook, P. M., and Olson, G. F. Ingested mineral fibers: elimination in human urine. Science 204: 195–198 (1979).
3. Hallenback, W. H., and Patel-Mandlik, K. J. Presence of fibers in the urine of a baboon gavaged with chrysotile asbestos. Environ. Res. 20: 335-340 (1979).
4. Patel-Mandlik, K. J., Hallenback, W. H., and Millette, J. R. Asbestos: A modified preparation of tissue samples for analysis by electron microscopy; 2. Presence of fibers in tissues of a baboon fed chrysotile asbestos. J. Environ. Pathol. Toxicol. 2: 1385–1395 (1979).
5. Patel-Mandlik, K.J., and Millette, J. R. Evidence of migration of ingested asbestos into various baboon organs. Scanning Electron Microscopy 1: 342–354 (1980).
6. Cunningham, H. M., and Pontefract, R. D. Asbestos fibers in beverages and drinking water and tissues: their passage through the intestinal wall and movement through the body. J. Assoc. Offc. Anal. Chem. 56: 976–981 (1973).
7. Cunningham, H. M., Moodie, C. A., Lawrence, G. A., and Pontefract, R. D. Chronic effects of ingested asbestos in rats. Arch. Environ. Contam. Toxicol. 6: 507–513 (1977).
8. Sebastien, P., Masse, R., and Bignon, J. Recovery of ingested asbestos fibers from the gastrointestinal lymph in rats. Environ. Res. 22: 201–216 (1980).
9. Boatman, E. Analyses of urines from residents drinking water containing asbestos in the Pacific Northwest. Paper presented at the U.S. Summary Workshop on Ingested Asbestos, Cincinnati, October 13-14, 1982.
10. Gross, P., Harley, R. A., Swinburne, L. M., Davis, J. M. G., and Greene, W. B. Ingested mineral fibers. Do they penetrate tissue or cause cancer? Arch. Environ. Health 29: 341–347 (1974).
11. Bolton, R. E., Davis, J. M. G., and Lamb, D. The pathological effects of prolonged asbestos ingestion in rats. Environ. Res. 29: 134–150 (1982).
12. Hallenback, W. H., Markey, D. R., and Dolan, D. G. Analyses of tissue, blood, and urine samples from a baboon gavaged with chrysotile and crocidolite asbestos. Environ. Res. 25: 349–360 (1981).
13. Bolton, R. E., and Davis, J. M. G. The short-term effect of chronic asbestos ingestion in rats. Ann. Occup. Hyg. 19: 121–128 (1976).
14. Auerbach, O., Hammond, E. C., Selikoff, I. J., Parks, V. R., Koslow, H. O., and Garfinkel, L. Asbestos bodies in lung

parenchyma in relation to ingestion and inhalation of mineral fibers. Environ. Res. 14: 286–304 (1977).
15. Storeygard, A. R., and Brown, A. L. Penetration of the small intestinal mucosa by asbestos fibers. Mayo Clin. Proc. 52: 809–812 (1977).
16. Westlake, G. E., Spjut, H. J., and Smith, M. N. Penetration of colonic mucosa by asbestos particles. Lab. Invest. 14: 2029–2033 (1965).
17. Jacobs, R., Humphrys, J., Dodgson, K. S., and Richards, R. J. Light and electron microscope studies of the rat digestive tract following prolonged and short-term ingestion of chrysotile asbestos. Brit. J. Exptl. Pathol. 59: 443–543 (1978).
18. Epstein, S. S., and Varnes, M. The short-term effects of ingested chrysotile asbestos on DNA synthesis in the pancreas and other organs of a primate. Experentia 32: 602–604 (1976).
19. Jacobs, R., Dodgson, K. S., and Richards, R. J. A preliminary study of biochemical changes in the rat small intestine following long-term ingestion of chrysotile asbestos. Brit. J. Exptl. Pathol. 58: 541–548 (1977).
20. Amacher, D. E., Alarif, A., and Epstein, S. S Effects of ingested chrysotile on DNA synthesis in the gastrointestinal tract and liver of the rat. Environ. Health Perspect. 9: 319–324 (1974).
21. Amacher, D. E., Alarif, A., and Epstein, S. S. The dose-dependent effects of ingested chrysotile on DNA synthesis in the gastrointestinal tract, liver and pancreas of the rat. Environ. Res. 10: 208–216 (1975).
22. Webster, I. The ingestion of asbestos fibers. Environ. Health Perspect. 9: 199–200 (1974).
23. Best, C. H., and Taylor, N. B. The Physiological Basis of Medical Practice, 8th ed. Williams and Wilkins, Baltimore-Toronto, 1967.

Environmental Health Perspectives
Vol. 53, pp. 153–154, 1983

Commentary

Asbestos Penetration of the Gastrointestinal Tract

by William H. Hallenbeck*

My comments are restricted to studies involving gastrointestinal exposure of humans and animals to asbestos via the diet or intubation. There have been three studies designed to determine whether human ingestion of asbestos in drinking water could result in the detection of asbestos in urine or postmortem tissues (*1–3*). One study concluded that ingested asbestos could not be found in human urine (*1*). However, other studies found evidence that ingested asbestos could penetrate the GI tract, migrate, and be recovered in urine and tissues (*2,3*). These two positive human studies were uncontrolled to the extent that inhaled asbestos may have contributed to the positive findings. Hence, we look to controlled animal studies for confirmation of the human findings, especially with regard to dose.

There have been at least 12 animal studies involving rats and baboons (*4–15*). Five rat studies (*4–8*) and two baboon studies (*8–10*) presented evidence of asbestos penetration of the GI tract and/or migration to various tissues. Three rat studies (*11–13*) and two baboon studies (*14, 15*) found little or no evidence of penetration and migration.

The positive animal findings (*4–10*) are not completely convincing for various reasons: (a) the number of fibers observed on a grid opening basis was quite small (*8, 10*); (b) the qualitative presentation of data prevented numerical comparisons of test and control fiber count data (*4,6*); (c) too few control data were obtained (*7*); and (d) fiber count data were reported in terms of fibers per milligram or milliliter (*5*). Unless fiber count data are given in terms of fibers per grid opening, it is not possible to know whether the authors' conclusions are based on the observation of one or hundreds of fibers. Given the problem of background contamination, positive results are always more credible if they are based on the actual observation of many fibers.

The negative animal studies are not completely convincing because the asbestos recovery of the sample preparation and analysis techniques were not demonstrated (*11–15*). It is possible that the methods used in these studies were not capable of detecting low levels of asbestos fibers in tissues.

Two additional studies are in progress in our laboratory. These will be completed in early 1983. Mark Finn is nearing completion of a study with two main objectives. The first objective is to compare three methods of sample preparation on the bases of accuracy, precision, and recovery. The three methods include the EPA interim methodology on ashed and unashed samples, condensation washing of unashed samples, and acetone-droplet transfer of ashed samples. Finn's second objective is to evaluate urine as a biological index of worker exposure to airborne asbestos (using the EPA provisional methodology).

Janet Kaczinski will complete a reevaluation of the baboon tissues reported on earlier (*15*). She is using the EPA provisional methodology instead of the acetone-droplet transfer technique used previously. Finn's research has shown that the recovery of the EPA provisional methodology is better than that of the acetone-droplet technique. Kaczenski has documented the recovery of her tissue preparation technique by analyzing asbestos-spiked tissue digests. Also, she is analyzing a relatively large amount of each tissue, 5 g wet weight.

The views and policies presented by the author in this commentary do not necessarily reflect those of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

*School of Public Health, University of Illinois, P.O. Box 6998, Chicago, IL 60680

REFERENCES

1. Boatman, E. S., Merrill, T., O'Neill, A., Polissar, L., and Millette, J. R. Use of quantitative analysis of urine to assess exposure to asbestos fibers in drinking water in the Puget Sound region. Environ. Health Perspect. 53: 129–139 (1983).
2. Cook, P. M., and Olson, G. F. Ingested mineral fibers: elimination in human urine. Science 204: 195–198 (1979).
3. Carter, R. E., and Taylor, W. F. Identification of a particular amphibole asbestos fiber in tissues of persons exposed to a high oral intake of the mineral. Environ. Res. 21: 85–93 (1980).
4. Westlake, G. E., Spjut, H. L., and Smith, M. N. Penetration of colonic mucosa by asbestos particles. Lab. Invest. 14: 2029–2033 (1965).
5. Cunningham, H. M., Moodie, C. A., Lawrence, G. A., and Pontefract, R. D. Chronic effects of ingested asbestos in rats. Arch. Environ. Contam. Toxicol. 6: 507–513 (1977).
6. Donham, K. J., Berg, J. W., Will, L. A., and Leininger, J. R. The effects of long-term ingestion of asbestos on the colon of F344 rats. Cancer 45: 1073–1084 (1980).
7. Sebastien, P., Masse, R., and Bignon, J. Recovery of ingested asbestos fibers from the gastrointestinal lymph in rats. Environ. Res. 22: 201–216 (1980).
8. Patel-Mandlik, K. J., and Millette, J. R. Chrysotile asbestos in kidney cortex of chronically gavaged rats. Arch. Environ. Contam. Toxicol. 12: 247–255 (1983).
9. Patel-Mandlik, K. J. Hallenbeck, W. H., and Millette, J. R. Asbestos fibers: 1. a modified preparation of tissue samples for analysis by electron microscopy. 2. presence of fibers in tissues of baboon fed chrysotile asbestos. J. Environ. Pathol. Toxicol. 2: 1385–1395 (1979).
10. Patel-Mandlik, K. J., and Millette, J. R. Evidence of migration of ingested asbestos into various baboon organs. Scanning Electron Microsc. 1: 347–354 (1980).
11. Gross, P., Harley, R. A., Swinburne, L. M., Davis, J. M. G., and Greene, W. B. Ingested mineral fibers: do they penetrate tissue or cause cancer? Arch. Environ. Health 29: 341–347 (1974).
12. Bolton, R. E., and Davis, J. M. G. The short-term effects of chronic asbestos ingestion in rats. Ann. Occup. Hyg. 19: 121–128 (1976).
13. Bolton, R. E., Davis, J. M. G., and Lamb, D. Pathological effects of prolonged asbestos ingestion in rats. Environ. Res. 29: 134–150 (1982).
14. Hallenbeck, W. H., and Patel-Mandlik, K. J. Presence of fibers in the urine of a baboon gavaged with chrysotile asbestos. Environ. Res. 20: 335–340 (1979).
15. Hallenbeck, W. H., Markey, D. R., and Dolan, D. G. Analyses of tissue, blood, and urine samples from a baboon gavaged with chrysotile and crocidolite asbestos. Environ. Res. 25: 349–360 (1981).

Environmental Health Perspectives
Vol. 53, pp. 115–120, 1983

Relative Source Contributions of Diet and Air to Ingested Asbestos Exposure

by James N. Rowe*

Reliable assessments of the relative contributions of diet (food, beverages, and orally administered drugs) and air (inhaled fibers) to total ingested asbestos exposure are not feasible due to the paucity of quantitative data on the subject. Instead, scenarios for both modes of exposure were developed from the limited information available to give crude estimates of ingestion of asbestos from these routes. They suggest that such sources are potentially significant relative to the contribution of asbestos exposure derived from drinking water. Research recommendations are discussed.

The general public has become aware of the potential gastrointestinal exposure to asbestos fibers from drinking water flowing through asbestos cement pipe or from fibers derived from natural sources of asbestos in rivers or reservoirs. However, most people are unaware that diet and air may also contribute significantly to the ingested asbestos exposure. The following is a discussion of the potential relative source contributions of diet, air and drinking water sources to ingested asbestos exposure. Information gaps in this area exist that preclude an accurate measure of the total ingested load of asbestos. The types of studies needed to fill these gaps are identified.

Asbestos in Dietary Materials

Only sparse data are available upon which one can estimate with any assurance the magnitude of asbestos exposure to the gastrointestinal tract from airborne and dietary sources, however, limited information is available for some sources that allow a crude measure of the ingested asbestos load to be calculated. Dietary materials that have been reported to contain, or are likely to contain, asbestos include foods such as vegetable oil, lard, mayonnaise, ketchup, meats (*1–3*) and beverages such as beers, sherries, ports, vermouth and soft drinks (*4–6*). In processed or filtered foods, the contamination is most likely due to the filtration process during which asbestos fibers are released into the processed materials. Other direct or indirect sources of asbestiform minerals in the food industry are in building materials, e.g., cement floor and ceiling tiles, pipe coverings and brake linings of transport vehicles (*7*). In addition, tremolite asbestos is found in talc-coated rice and chewing gum, as well as in oral drugs containing talc as an incipient in compressed tablets, as a dusting powder in capsules, and, less frequently, as a filler in the capsules (*8*).

Contamination by asbestos is so common that many, if not most, foods may contain some asbestos contamination (P. McGrath, FDA, personal communication). Unfortunately, there is presently no established program to examine exhaustively dietary materials for their asbestos content.

Limited, but important, quantitative data on the concentrations of asbestos in ingested sources are given in Table 1. In alcoholic and nonalcoholic beverages, Cunningham and Pontefract (*5*) reported asbestos concentrations ranging from 1.1 to 12.2 $\times$ 10^6 fibers/L as measured by the transmission electron microscope (TEM). The asbestos content of a sample of talc-coated rice destined for Japanese consumers was estimated by Merliss (*1*) to contain 3.7 $\times$ 10^6 fibers/g as measured through the light optical microscope (LOM). The asbestos concentration in drugs, including three brands of aspirin, has been determined by Nicholson (*9*) to range from 120 to 150 ng/g.

Using this information, a scenario could be constructed along the following lines: John Q. Public is an "average" individual who daily

*Office of Toxic Substances (TS-796), U.S. Environmental Protection Agency, 401 M Street, SW, Washington, DC 20460.

Environmental Health Perspectives
Vol. 53, pp. 155–161, 1983

In Vitro Approaches for Determining Mechanisms of Toxicity and Carcinogenicity by Asbestos in the Gastrointestinal and Respiratory Tracts

by Brooke T. Mossman*

Organ and cell cultures of gastrointestinal and tracheobronchial epithelium have been used to document both the interaction of asbestos with mucosal cells and the sequence of cellular events occurring after exposure of cells to fibers. The biological activity of various types of asbestos *in vitro* is related to surface charge, crystallization, and dimensional characteristics. These factors also influence adsorption of natural secretions and serum components to fibers, a process that ameliorates cytotoxicity. Although mechanistic studies at the cellular level are lacking using epithelial cells of the digestive tract, asbestos appears to elicit a constellation of morphologic and biochemical changes in tracheal epithelium that resemble effects of classical tumor promoters on target cells.

Introduction

Asbestos is implicated in the causation of cancers of the digestive system, airways, and lung. To elucidate possible mechanisms of fiber-induced disease, the interaction of asbestos with epithelial cells of the gastrointestinal and respiratory tracts has been studied in organ and cell cultures. After introduction of asbestos, the uptake of fibers by mucosal cells is observed concomitantly with damage, death, and regeneration of the epithelium (*1–3*). The extent of these changes, which might be related to the process of carcinogenesis, varies with the type (*2–4*), charge (*5,6*), crystallization (*7*), and size (*8,9*) of the fibers. Discussed here are experiments *in vitro* that define asbestos-induced cellular responses and their possible relationship to neoplasia in gastrointestinal and respiratory epithelium. Carcinoma, the histologic classification of tumor found most frequently in man, arises from these (i.e., epithelial) cells. Although only one group of researchers has taken an in-depth look at the testing of asbestos in gastrointestinal epithelial cells *in vitro* (*4*), the work done with other epithelial cells provides significant data.

*Department of Pathology, University of Vermont, College of Medicine, Burlington, VT 05405.

Mechanisms of Cell Damage by Asbestos

Because asbestos is a family of fibrous hydrated silicates, each with unique chemicophysical features, the relative toxicity of various types of asbestos has been assessed comparatively in cell and organ cultures derived from intestinal, liver, colonic (*4*), and tracheobronchial epithelium (*2,3,10,11*). Cell damage, compared to untreated controls, is assessed by inhibition of growth or colony formation (*2,4*), release of ^{51}Cr (*10*) or ^{75}Se (*11*) from prelabeled cultures, and quantitation of lysosomal or cytoplasmic enzymes in medium (*12*). Although the relative sensitivity of different cell lines varies, chrysotile is more toxic when compared at equal concentrations to the amphiboles, amosite and crocidolite.

Surface Charge and Cytotoxicity

To elucidate mechanisms of asbestos-induced cytotoxicity, the chemical and structural composition of asbestos has been altered experimentally by leaching and adsorption of macromolecules.

These changes are thought to occur after inhalation or ingestion of fibers *in vivo*. For example, unlike a soluble material of defined chemical composition, asbestos is insoluble and comprised of a number of extractable elements that are removed after prolonged periods of time *in vivo* (*13–15*).

After incubation of chrysotile in hydrochloric acid, substantial amounts of cations, including Mg^{2+} and Ca^{2+}, are released from fibers (*4*). Accordingly, the surface charge of fibers, as measured by the zeta potential, is altered. Experiments by Light and Wei (*5,6*) have indicated the important role of surface charge in asbestos-induced toxicity by quantitating hemolysis in red blood cells (RBC) after adding natural and leached asbestos.

Under physiological conditions, the surface charges of chrysotile and crocidolite asbestos are similar in magnitude, but opposite in polarity (+44.5 mV for chrysotile and −43.5 mV for crocidolite). Chrysotile causes distortion and hemolysis of RBC, whereas crocidolite is only weakly hemolytic. After leaching, however, the surface charge of chrysotile decreases as does its hemolytic activity. In contrast, the hemolytic potential for crocidolite increases proportionately as it becomes more negative in charge. Leaching chrysotile in hydrochloric acid also inhibits its cytotoxicity in epithelial cells from liver (*4*) and trachea (*10*) while cytotoxicity of leached crocidolite is enhanced.

In additional experiments by Light and Wei (*6*), dipalmitoyl phosphatidylcholine (DPPC), the main component of pulmonary surfactant, a substance secreted by the lung, was incubated with unleached fibers before their addition to RBC. Under these circumstances, DPPC decreases the surface charge of both chrysotile and crocidolite with a proportional inhibition of hemolysis. Thus, asbestos fibers appear to adsorb macromolecules that can ameliorate their cytotoxicity. These results are supported by those of Desai and Richards (*16*) showing a selective adsorption and retention of serum proteins by chrysotile and crocidolite after incubation in culture medium. The addition of fibers to cell cultures in serum, versus medium containing no serum, also decreases the growth-inhibitory effects of asbestos (*2*)(Fig. 1).

Fiber Size and Cytotoxicity

Addition of asbestos to a number of cell types in culture is associated with release of lysosomal and cytoplasmic enzymes into the medium (*12,17*). In macrophagelike cells, the amount of cell contact and phagocytosis correlate directly with a decrease in number of viable cells, whereas cultures of fibroblasts, a relatively nonphagocytic cell type, are more resistant to asbestos (*18*). The dimensions of the fiber also determine how the cell will react. For example, toxicity is reduced substantially when larger fibers and particles are reduced to a submicron size range (*8,9*). In general, shorter, smaller fibers can be phagocytized, whereas macrofibers cannot be enveloped completely. Under the latter circumstances, one might postulate that damage to membranes results in cell death, and remnants of cellular debris can be seen in association with fibers that are phagocytized incompletely (Fig. 2). Recent data from our laboratory suggest that oxygen-free radicals are generated when longer fibers are encompassed by tracheal epithelial cells (*11*). Moreover, levels of superoxide dismutase (SOD), an enzyme that converts the superoxide radical (O_2^- to H_2O_2 and O_2, appear to be altered in cells after exposure to asbestos. More importantly, cell damage by longer fibers can be prevented by addition of scavengers of oxygen-free radicals to cultures.

Surface Area and Crystallization

The mechanisms of cell lysis by asbestos are also related to specific surface area (*19*) and crystallization of the fiber (*7*). Dimensional characteristics appear important in determining the amount of cell contact, whereas the role of structure is enigmatic. Palekar and colleagues compared the hemolytic and cytotoxic potential of structurally modified forms of amosite, an amphi-

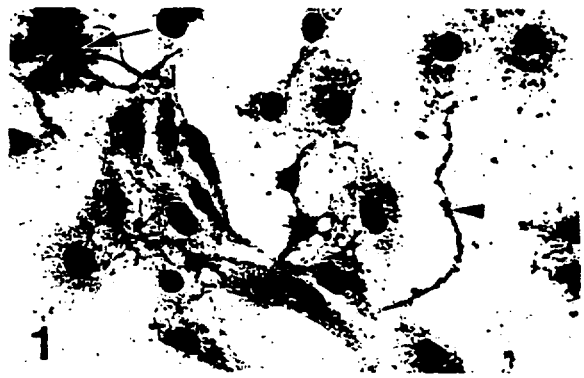


FIGURE 1. When chrysotile asbestos (5 μg/mL F12 medium, 10% calf serum) is added to monolayer cultures of tracheal epithelial cells, cytotoxic alterations such as sloughing of cells, multinucleated cells (arrow), and prominent cytoplasmic granules are observed. The adsorption of basic dye appears as beads on the fiber (arrowhead). Giemsa, ×1035.

bole asbestos (7). These minerals occurred in various developmental stages of crystallization and included both species with structural and surface defects and more "asbestiform" varieties, i.e., long, perfect fibers with high tensile strength. All samples were characterized for surface area, charge and chemical content. When assayed at comparable surface areas, hemolytic activity and cytotoxicity in cell cultures were related directly to the degree of crystallization. Amosite exhibited the greatest biological activity, whereas the most nonasbestiform mineral was inert.

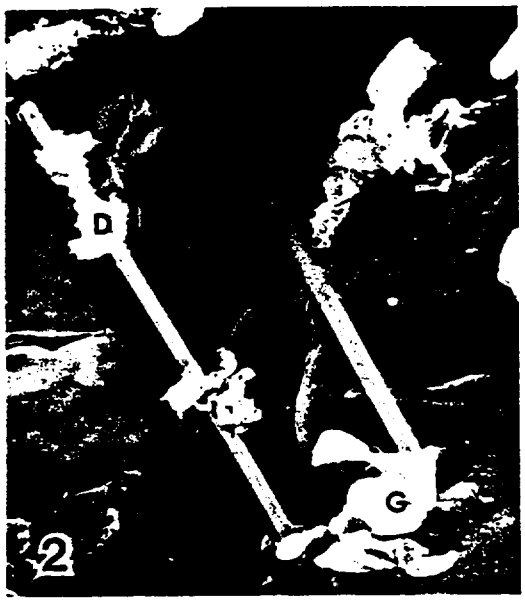
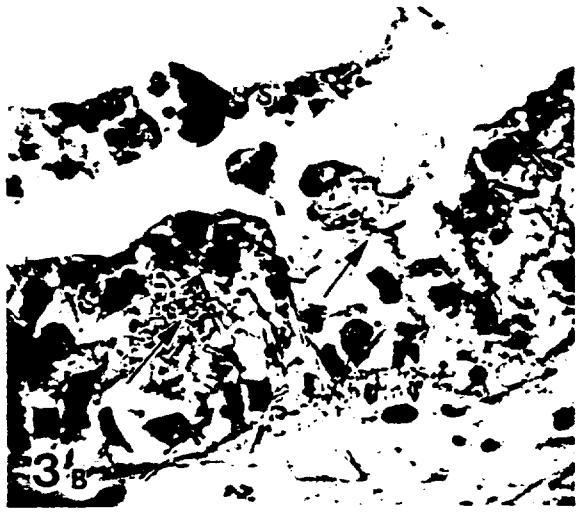


FIGURE 2. Larger fibers (i.e., >10 μm length) are phagocytized incompletely by human bronchial epithelial cells, and are more cytotoxic than smaller fibers (i.e., <2 μm) *in vitro*. Note the cellular debris (D) and cell ghost (G) in association with the fiber. This scanning electron micrograph was furnished by Craig Woodworth, Department of Pathology, UVM College of Medicine, ×560.

Injury, Promotion and Carcinogenesis

After injury and death of epithelial cells of the gastrointestinal and respiratory tracts, luminal cells are sloughed and replaced by basal cells, the presumed progenitors of carcinoma (Fig. 3). Be-



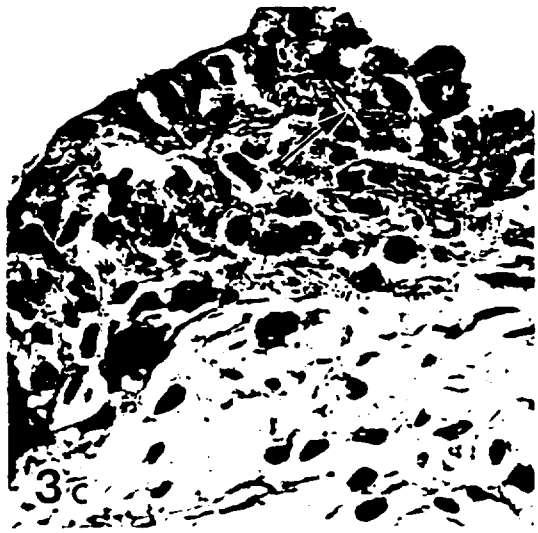


FIGURE 3. Sequential morphologic changes in organ cultures of hamster tracheal epithelium after exposure to crocidolite asbestos. Tissues are exposed to asbestos for 1 hr before transfer to asbestos-free medium for extended periods of time: (*A*) culture without addition of asbestos at 1 week after preparation, ×1400; (*B*) at 72 hr after addition of crocidolite (arrows, 4 mg/mL medium), sloughing of superficial cells (S) occurs, ×1600; (*C*) a hyperplastic lesion is observed at 1 week after addition of asbestos. Note the accumulation of refractile crocidolite (arrow) within the mucosa, ×1400. Hematoxylin and eosin.

cause malignant transformation of cells occurs specifically during the DNA synthetic phase of the cell cycle (*20*), asbestos-induced proliferation of cells might be an intrinsic mechanism of carcinogenesis. In the next several paragraphs, the concepts of initiation and promotion in carcinogenesis will be discussed. Moreover, we will describe the properties of asbestos that suggest its role as a tumor promoter in the development of gastrointestinal and bronchogenic carcinomas. The experiments in our laboratory have been performed using epithelial cells from the respiratory tract; however, since these cells are similar structurally and functionally to mucin cells of the gut, our results might apply to events occurring in the gastrointestinal mucosa after ingestion of asbestos.

Initiation and Promotion

The process of carcinogenesis can be dissected into sequential stages of initiation and promotion (*21*). An initiator is defined as an agent interacting directly with the DNA of a cell, whereas a promoter influences the establishment and develoment of a tumor. As discussed by Daniel in these proceedings (*22*), the evidence implicating asbestos as a mutagenic agent in intestinal and other cell types is weak; thus its role as an initiator of carcinogenesis is questionable. On the other hand, work from our laboratory shows that the properties of asbestos in cell and organ cultures of trachea are similar to classical tumor promoters such as phorbol esters (*23, 24*). These substances cause proliferative and biochemical alterations in cells including: (a) attachment to and entrance into target cells; (b) stimulation of cellular division; (c) inhibition of normal cell differentiation; and (d) increased activity of ornithine decarboxylase (ODC), the rate-limiting enzyme in the biosynthesis of polyamines.

Uptake of Asbestos by Mucosal Cells

Fibers penetrate intestinal and respiratory mucosa both *in vivo* and *in vitro* (*1, 25, 26*) and remain in epithelial cells for extended periods of time (Fig. 4 and 5). These processes not only allow continuous cellular exposure to the dust, but also permit the access into cells of chemical carcinogens adsorbed to the fibers. As an example, organic material, including carcinogenic polycyclic aromatic hydrocarbons (PAH), are natural contaminants of asbestos (*27*).

In experimental systems, the association of PAH with the fiber surface appears to enhance cellular uptake of the hydrocarbon. Thus, asbestos might be cocarcinogenic by facilitating the entrance of documented chemical carcinogens into cells. For example, Lakowicz and colleagues (*20, 29*) document by fluorimetry the increased transfer of PAH to artificial membranes when chemicals are adsorbed to asbestos. On the other

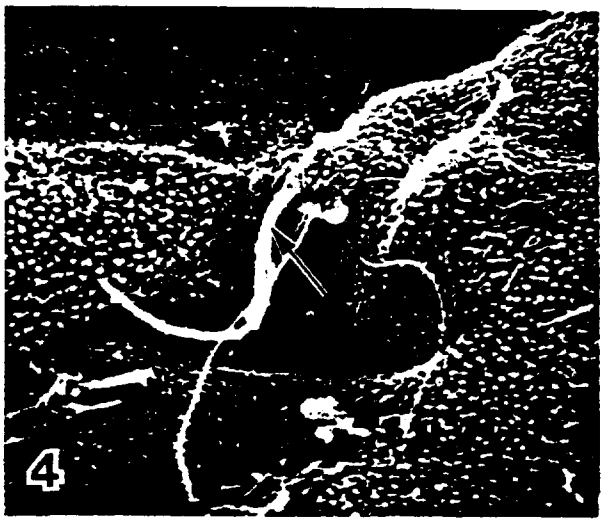


FIGURE 4. Human bronchial explant 24 hr after exposure for 1 hr to chrysotile asbestos (1 mg/mL medium). Note the long fibers (arrows) protruding from the epithelial cells. This scanning electron micrograph was furnished by Craig Woodworth, Department of Pathology, UVM College of Medicine, ×6120.

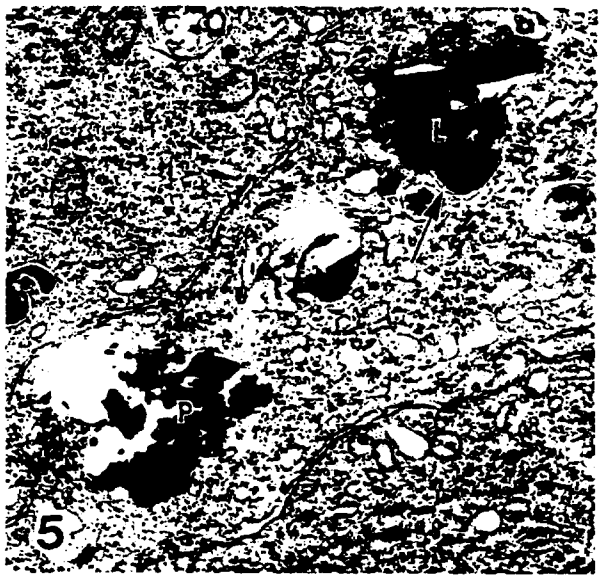


FIGURE 5. Transmission electron micrograph of crocidolite asbestos in a basal epithelial cell of hamster trachea. The asbestos occurs in membrane-bound (arrow) phagosomes (P) and phagolysosomes (L). Uranyl acetate and lead citrate, ×7500.

hand, dispersions and particles of PAH alone do not transfer rapidly to membranes.

When ^{3}H-BaP is adsorbed either to crocidolite or chrysotile asbestos before its addition to cultures of tracheal epithelial cells, approximately 70% of the total BaP introduced enters the cell within 1 hr., whereas 50% remains intracellular and unmetabolized at 8 hr. (*30*). In contrast, if identical amounts of BaP are added directly to medium, an influx of only 20% is observed and cells retain 5% of the initial amount at 8 hr. Under the former conditions, alkylation of BaP to DNA is increased for as long as 5 days after introduction of asbestos adsorbed to BaP. Increased uptake, retention, and alkylation of BaP to DNA are not observed when BaP is added 1 hr. after the asbestos (*31*).

Stimulation of Cell Replication by Asbestos

After exposure to amosite or crocidolite asbestos, tracheal epithelial cells *in vitro* show increased incorporation of ^{3}H-thymidine, an indication of DNA synthesis, and basal cell hyperplasia (*32*). Although enhanced uptake of ^{3}H-thymidine and morphologic changes are also observed in monolayers of cells exposed to either crocidolite of chrysotile (*33*), proliferative alterations appear more transiently in organ cultures exposed to the latter type of asbestos.

An increase in cell replication could contribute in several ways to carcinogenesis. First, it would give an "initiated" cell (i.e., one that is committed to becoming malignant) a selective advantage over normal cells. Second, since cells are transformed by chemical carcinogens only during DNA synthesis, a cell preparing for division is more susceptible to transformation.

Induction of Squamous Metaplasia by Asbestos

Squamous metaplasia is defined as the replacement of a normal mucin-secreting epithelium by keratinizing squamous cells. This lesion, although reversible, can be considered preneoplastic. The appearance of squamous metaplasia in the gastrointestinal or respiratory tract could be a promoting influence in carcinogenesis by the following mechanisms. First, mucin secretion by differentiated epithelial cells is interrupted, thus removing the protective barrier against asbestos and other carcinogens.

Second, the interaction and uptake of fibers by squamous epithelium are observed frequently *in vitro* (Fig. 6) (*34*); therefore, increased entrance into, and retention of asbestos by, squamous epithelium is a possibility. Last, the basal cells of squamous lesions are actively dividing (*32*), a process encouraging their transformation by carcinogens in general.

Stimulation of Ornithine Decarboxylase (ODC) by Asbestos

The magnitude of induction of ODC, the first and rate-limiting enzyme in the biosynthesis of polyamines, relates directly to the tumor-promoting capabilities of a number of phorbol compounds in mouse skin (*35*). When chrysotile or crocidolite is added to tracheal epithelial cells, ODC is increased in a dosage-dependent fashion; however, the magnitude and latency of response differ from that observed with the phorbol, 12-tetradecanoylphorbol-13-acetate (TPA) (*33*). The increase in enzyme induction occurs concomitantly with a mitogenic response as measured by uptake of ^{3}H-thymidine.

An increased synthesis of polyamines might be important in carcinogenesis because it is linked intrinsically to cell division. For example, critical cellular levels of polyamines must exist for cell division to occur.

FIGURE 6. Surface of a hamster tracheal explant at 4 weeks after addition of amosite asbestos (4 mg/mL medium). The superficial cells are becoming squamous (S). Note the fiber within the cell (arrow) and the fiber (arrowhead) on the epithelial surface. Scanning electron micrograph, ×2000.

Summary and Conclusions

Asbestos interacts with mucosal cells of the gastrointestinal and respiratory tracts, although its composition and cytotoxicity are modified by acidity and coating with natural secretions such as mucin and surfactant. The biological activity of the fibers is determined by both surface charge, crystallization, and dimensional characteristics. After damage and death of superficial cells of the respiratory tract, replacement occurs via actively dividing basal cells. These latter cell types become squamous and exhibit increased synthesis of polyamines.

The role of asbestos in carcinogenesis appears epigenetic and can be compared to that of a classical tumor promoter. A number of experimental and epidemiologic studies support this observation. For example, various types of asbestos do not cause single-strand breakage of DNA (*30*) and are not mutagenic in a number of cell types (*22*). Moreover, asbestos, unless combined with PAH, is not carcinogenic in hamster tracheal implants (*36,37*) and is only weakly carcinogenic in rats after repeated intratracheal instillations (*23*).

Compelling epidemiologic evidence also indicates that asbestos acts synergistically with cigarette smoke to induce tumors of the respiratory tract. Persons occupationally exposed to asbestos (smokers and nonsmokers as a group) have an eightfold higher incidence of bronchogenic carcinoma than the general population. While the increase (1.5- to 4-fold) in disease among nonsmoking asbestos workers is small, smokers have an 80- to 90-fold greater predisposition (*38,39*).

Although asbestos acts like a promoting agent in the airways and lungs, there is little experimental evidence documenting this capability in the gastrointestinal tract. Mechanistic studies to evaluate the promoterlike activity of asbestos in organ and cell culture of esophagus, stomach, and/or intestine should be encouraged.

The author acknowledges the excellent technical assistance of Joan Carrassi, Bettie Clements, Lucy Jean, and Joanne Marsh in the experimental studies by the author described here. This research was supported by grant RO1 OH00888 from the National Institute for Occupational Safety and Health, grant PHS RO1 33501 from the National Cancer Institute, and grant BC-415 from the American Cancer Society. Preparation of this manuscript was supported by Contract No. 68-03-3130 from the U.S. Environmental Protection Agency.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Mossman, B. T., Kessler, J. B., Ley, B. W., and Craighead, J. E. Interaction of crocidolite asbestos with hamster respiratory mucosa in organ culture. Lab. Invest. 36: 131–139 (1977).
2. Mossman, B. T., Adler, K. B., and Craighead, J. E., Cytotoxic and proliferative changes in tracheal organ and cell cultures after exposure to mineral dusts. In: The *In Vitro* Effects of Mineral Dusts (R. Brown, I. P. Gormley, M. Chamberlain and R. Davies, Eds.), Academic Press, London, 1980, pp. 241–250.
3. Craighead, J. D., Bradley, B. J., and Mossman, B. T. Comparative studies on the cytotoxicity of amphibole and serpentine asbestos. Environ. Health Perspect. 34: 37–46 (1980).
4. Reiss, B., Solomon, S., Weisburger, W., and Williams, G. Comparative toxicities of different forms of asbestos in a cell culture assay. Environ. Res. 22: 109–129 (1980).
5. Light, W. G., and Wei, E. T. Surface charge and asbestos toxicity. Nature 265: 537–539 (1977).
6. Light, W. G., and Wei, E. T. Surface charge and hemolytic activity of asbestos. Environ. Res. 13: 135–145 (1977).
7. Palekar, L. D., Spooner, C. M., and Coffin, D. L. Influence of crystallization habit of minerals on *in vitro* cytotoxicity. Ann. N.Y. Acad. Sci. 330: 673–686 (1979).
8. Kaw, J. E., Tilkes, F., and Beck, E. G. Reaction of cells cultured *in vitro* to different asbestos dusts of equal surface area but different fibre length. Brit. J. Exptl. Pathol. 63: 109–115 (1982).
9. Brown, R. C., Chamberlain, M., Griffiths, D. M., and Timbrell, V. The effects of fibre size on the in vitro biological activity of three types of amphibole asbestos. Int. J. Cancer 22: 721–727 (1978).
10. Woodworth, C. D., Mossman, B. T., and Craighead, J. E. Comparative effects of fibrous and nonfibrous minerals on cells and liposomes. Environ. Res. 27: 190–205 (1982).
11. Mossman, B. T., and Landesman, J. M. Importance of oxygen free radicals in asbestos-induced injury to airway epithelial cells. Proc. 25th Aspen Lung Conf., Chest 835: 505–515 (1983).
12. Davies, P., Allison, A. C., Ackerman, J., Butterfield, A., and Williams, S. Asbestos induces selective release of lysosomal enzymes from mononuclear phagocytes. Nature 251: 423–425 (1974).
13. Morgan, A., Holmes, A., and Gold, C. Studies of the solubility of constituents of chrysotile asbestos *in vivo* using radioactive tracer techniques. Environ. Res. 4: 558–570 (1971).
14. Langer, A. M., Rubin, J. B., Selikoff, I. J., and Pooley, F. D. Chemical characterization of uncoated asbestos fibers from the lungs of asbestos workers by electron microprobe analysis. J. Histochem. Cytochem. 20: 735–740 (1972).
15. Jaurand, M. C., Bignon, J., Sebastien, P., and Goni, J. Leaching of chrysotile asbestos in human lungs. Correlation with *in vitro* studies using rabbit alveolar macrophages. Environ. Res. 14: 245–254 (1979).
16. Desai, R., and Richards, R. J. The adsorption of biological macro-molecules by mineral dusts. Environ. Res. 16: 449–464 (1978).
17. Jaurand, M. C., Magne, L., Boulmer, J-L., and Bignon, J. *In vitro* reactivity of alveolar macrophages and red blood cells with asbestos fibres treated with oxalic acid, sulfur dioxide and benzo-3,4-pyrene. Toxicology 21: 323–342 (1981).
18. Wade, M. J., Lipkin, L. E., and Frank, A. L. Studies of *in vitro* asbestos-cell interaction. J. Environ. Pathol. Toxicol. 2: 1029–1039 (1979).

19. Morgan, A. Adsorption of human serum albumin by asbestiform minerals and its application to the measurement of surface areas of dispersed samples of chrysotile. Environ. Res. 7: 330–341 (1974).
20. Marquardt, H. Cell cycle dependence of chemically induced malignant transformation *in vitro*. Cancer Res. 34: 1612–1619 (1974).
21. Berenblum, I. Historical perspective. In: Carcinogenesis, A Comprehensive Survey (T. J. Slaga, A. Sivak and R. K. Boutwell, Eds.), Raven Press, New York, 1978, pp. 1–13.
22. Daniel, F. B. *In vitro* assessment of asbestos genotoxicity. Environ. Health Perspect. 53: 161–165 (1983).
23. Mossman, B. T., and Craighead, J. E. Mechanisms of asbestos carcinogenesis. Environ. Res. 25: 269–280 (1981).
24. Mossman, B. T., Light, W. G., and Wei, E. T. Asbestos: Mechanisms of toxicity and carcinogenicity in the respiratory tract. Ann. Rev. Pharmacol. Toxicol. 23: 595–615 (1983).
25. Westlake, G. E., Spjut, H. A., and Smith, M. N. Penetration of colonic mucosa by asbestos particles. Lab. Invest. 14: 2029–2033 (1965).
26. Suzuki, Y. Interaction of asbestos with alveolar cells. Environ. Health Perspect. 9: 241–252 (1974).
27. Harington, J. S. Occurrence of oils containing 3:4-benzpyrene and related substances in asbestos. Nature 193: 43–45 (1962).
28. Lakowicz, J. R., and Bevan, D. R. Benzo(a)pyrene uptake into rat liver microsomes: effects of adsorption of benzo(a)pyrene to asbestos and non-fibrous mineral particulates. Chem.-Biol. Interact. 29: 129–138 (1980).
29. Lakowicz, J. R., McNamara, M., and Steenson, L. Particle-mediated membrane uptake of chemical carcinogens studied by fluorescence spectroscopy. Science 199: 305–306 (1978).
30. Mossman, B. T., Eastman, A., Landesman, J. M., and Bresnick, E. Effects of crocidolite and chrysotile asbestos on cellular uptake, metabolism and DNA after exposure of hamster tracheal epithelial cells to benzo(a)pyrene. Environ. Health Perspect., in press.
31. Eastman, A., Mossman, B. T., and Bresnick, E. The influence of asbestos on the uptake of benzo(a)pyrene and DNA alkylation in hamster tracheal epithelial cells. Cancer Res. 43: 1251–1255 (1983).
32. Mossman, B. T., Craighead, J. E., and MacPherson, B. V. Asbestos-induced epithelial changes in organ cultures of hamster trachea: inhibition by retinyl methyl ether. Science 207: 311–313 (1980).
33. Landesman, J. M., and Mossman, B. T. Induction of ornithine decarboxylase in hamster tracheal epithelial cells exposed to asbestos and 12-O-tetradecanoylphorbol-13-acetate. Cancer Res. 42: 3669–3675 (1982).
34. Woodworth, C. D., Mossman, B. T., and Craighead, J. E. Squamous metaplasia of the respiratory tract—possible pathogenic role in asbestos-associated bronchogenic carcinoma. Lab. Invest. 48: 578–584 (1983).
35. O'Brien, T. G. The induction of ornithine decarboxylase as an early, possibly obligatory, event in mouse skin carcinogenesis. Cancer Res. 36: 2644–2653 (1976).
36. Mossman, B. T., and Craighead, J. E. Use of hamster tracheal organ cultures for assessing the cocarcinogenic effects of inorganic particulates on the respiratory epithelium. Prog. Exptl. Tumor Res. 24: 37–47 (1978).
37. Mossman, B. T., and Craighead, J. E. Comparative cocarcinogenic effects of crocidolite asbestos, hematite, kaolin, and carbon in implanted hamster tracheal organ cultures. In: Inhaled Particles, Vol. 5 (W. H. Walton, Ed.), Ann. Occup. Hyg., Vol. 26, Pergamon Press, London, 1982, pp. 572–585.
38. Selikoff, I. J., Lilis, R., and Nicholson, W. J. Asbestos disease in United States shipyards. Ann. N.Y. Acad. Sci. 330: 295–311 (1980).
39. Selikoff, I. J., Seidman, H., and Hammond, E. C. Mortality effects of cigarette smoking among amosite asbestos factory workers. J. Natl. Cancer Inst. 65: 507–513 (1980).

Environmental Health Perspectives
Vol. 53, pp. 163–167, 1983

In Vitro Assessment of Asbestos Genotoxicity

by F. B. Daniel*

Asbestos fibers are highly cytotoxic to cultured mammalian cells and produce chromosomal aberrations in several rodent cell types. There is some uncertainty in the literature as to whether these fibers are clastogenic to cultured human cells. Asbestos fibers do not produce either DNA damage or back mutations in prokaryotic assay systems, and they do not appear to cause DNA strand breaks in either rodent or human cells. The evidence that these fibers can produce either forward mutation or neoplastic transformation of mammalian cells is weak. Asbestos fibers are clearly oncogenic to humans and animals, but, except for clastogenic effects in rodent cells, there is little evidence for genotoxicity of fibers. It is reasonable to expect, therefore, that these materials may be oncogenic by virtue of mechanisms rather than as tumor initiators.

Exposure to asbestos fibers has been shown to induce at least two types of carcinogenic response in the human respiratory system: mesothelioma of the pleural cavity (*1, 2*) and bronchial carcinoma (*1, 3*). Various forms of asbestos and other mineral fibers also induce malignant mesenchymal neoplasms in experimental animals (*4, 5*). At present it is unclear how these materials (often called solid-state carcinogens) initiate neoplastic responses in mammalian systems. However, it is important that our understanding of this phenomenon be improved, since a relatively large segment of the human population is exposed both occupationally (*1, 6*) and otherwise to asbestos and other mineral fibers. Comprehensive reviews on the physical, chemical, and biological properties of asbestos fibers have appeared (*7–9*).

In recent years considerable progress has been made in understanding the process of chemical carcinogenesis. It is now clear that chemicals can cause or facilitate oncogenesis by more than one mechanism (*10*). For example, many organic chemicals are carcinogenic only subsequent to their conversion, by cellular enzymes, to electrophilic species that can then react with cellular nucleophiles including various sites on the DNA molecule (*11*). The modified DNA is thus considered "damaged," and chemicals that effect such reactions are generically labeled genotoxins. While the type of DNA damage produced by these agents varies with the chemical properties of the genotoxin involved, it is widely believed that such chemical–DNA interactions are (or can be) initiating factors in chemical carcinogenesis. Further, the concept that tumor initiators are genotoxins has spawned the rapid development of a large number of "short-term" tests (many *in vitro* and, for the most part, based either directly or indirectly on the assessment of DNA damage), since lifetime carcinogenicity studies in experimental animals are both cost and labor intensive. This paper reviews the evidence that asbestos fibers are genotoxic as measured by various short-term test methodologies. For specifics on the various test procedures discussed, the reader is directed to several recent monographs (*12, 13*).

While the term asbestos will be used in the generic sense in this discussion, the reader should recall that it refers to a large group of fibrous minerals with varied chemical and physical properties, both of which can affect the carcinogenic potential of the material (*14–16*). Nevertheless, standard reference fibers of several asbestos types have been prepared by two scientific agencies, the Union Internationale Contra Cancer (UICC) and the National Institute of Environmental Health Sciences (NIEHS), for the purpose of providing continuity between scientific investigations conducted in various laboratories throughout the world. Since both of these fiber preparations have been thoroughly characterized (*17, 18*), this review will deal primarily with studies conducted on them.

*Health Effects Research Laboratory, U.S. Environmental Protection Agency, 26 West St. Clair St., Cincinnati, OH 45268.

Mossman et al. (*19*) have shown that asbestos fibers (UICC crocidolite and UICC chrysotile) do not produce DNA strand breaks in the alkaline elution assay when applied to cultured hamster tracheal cells. Recently, Lechner et al. (*20*) have obtained similar negative results with respect to the induction of DNA strand breakage in human bronchial organ cultures treated with UICC chrysotile, amosite, and crocidolite. These fibers were highly cytotoxic to both cell types (*19, 20*). Finally, a comprehensive study by Hart et al. (*21*) on a series of mineral fibers, including the NIEHS reference fibers, revealed no evidence of DNA damage in a series of assays including: unscheduled DNA synthesis in human fibroblasts, endonuclease sensitive sites, single-strand breaks (alkaline sucrose gradient sedimentation), or double-strand breaks (neutral sucrose gradient sedimentation).

Chamberlain and Tarmy (*22*) tested a number of mineral fibers (including the UICC reference standards: Canadian chrysotile B, amosite, anthophyllite, and crocidolite, a sample of SFA chrysotile, a superfine Canadian chrysotile, and two samples of fiber glass) for their ability to produce back mutations in *Salmonella typhimurium* and DNA damage in *Escherichia coli* WP2 bacteria. All of the preparations were inactive (maximum dose: 500 μg/plate) in both systems even when a rat liver supernate was employed as the metabolic activation system (*22*). The lack of a positive response could be, at least in part, ascribed to the fact that asbestos fibers are not taken up by bacterial cells (*22*). Similar results with respect to the effect of asbestos fibers on *S. typhimurium* were obtained by Light and Wei (*23*), who reported the neither UICC Canadian chrysotile B nor UICC crocidolite (maximum dose: 500 μg/plate) reverted any of the five most common tester strains to histidine independence. Thus it is tentatively concluded that mineral fibers do not produce genotoxic effects in prokaryotic systems.

In contrast to bacteria, cultured mammalian cells such as lung macrophages (*24*) or Chinese hamster ovary (CHO) cells (*25*) readily phagocytize asbestos and other mineral particles. In addition, asbestos fibers are extremely cytotoxic to many types of mammalian cells (*14, 26*).

An initial study by Kaplan et al. (*27*) indicated that treatment of cultured rat pleural mesothelial cells with up to 5 μg/mL of UICC Rhodesian chrysotile A for periods up to 32 hr did not elevate the level of sister chromatid exchanges (SCE) above that of control cells. However, a subsequent study (*28*) using CHO cells revealed that UICC crocidolite and UICC amosite did produce very slight (but significant) increases in SCE levels in cells treated for 64 hr at 10 μg/mL of fiber. The crocidolite seemed more effective than the amosite (which was ruled positive solely on the basis of a statistically significant decrease in number of cells exhibiting no SCE relative to the untreated control), and the SCE seemed confined predominantly to the larger chromosomes (*28*). A preparation of UICC chrysotile was too cytotoxic to permit SCE analysis even at the 10 μg/mL level (*28*). In contrast, a study by Price-Jones et al. (*29*) using Chinese hamster V79-4 cells showed that neither UICC crocidolite nor Min-U-Sil silica produced SCE even when applied at levels up to 15 μg/mL for up to 30 hr. Under this protocol, potassium chromate (500 μg/mL) was positive as an inducer of SCE (*29*). However, the higher doses of both fibers did cause statistically significant increases in the level of chromosome aberrations (aneuploids and polyploids) in the V79-4 cells (*29*). Taken together, these studies indicate that the UICC asbestos fibers are either negative or extremely weak as to their ability to produce SCE in mammalian cells.

Sincock and Seabright (*30*) first demonstrated that both chromatid and chromosomal changes occurred in CHO cells exposed to SFA Canadian chrysotile and UICC crocidolite but observed no aberrations in cells exposed, under an identical protocol (10 μg/mL, 48 hr), to preparations of glass fiber and glass powder. Identical results were obtained by exposing the cells to the fibers for 5 days before the cytogenetic analysis (*30*). If the fibers were preexposed to complete culture media (with serum), their clastogenic potential was reduced (*30*). Lavappa et al. (*31*) reported that a sample of Rhodesian chrysotile A (probably UICC) produced chromosomal aberrations (primarily chromatid breaks) in a dose-related manner in a Syrian hamster embryo (SHE) when applied at 0.1 to 100 μg/mL for 6 to 96 hr. The preponderance of chromatid damage led the investigators to propose that the G_2 was the most sensitive phase of the mitotic cycle to asbestos fiber clastogenic effects (*30*). It is noteworthy that these same fiber preparations, when administered *in vivo*, did not induce either chromosomal aberrations (monkey, oral administration) or micronuclei (mouse, intraperitoneal administration) in bone marrow cells (*31*). Finally, the induction of chromosome aberrations following administration of crocidolite (origin unspecified) to Chinese hamster lung cells has been observed by Huang et al. (*25*). In these studies the cytotoxic properties of the fibers were associated with their engulf-

ment by the cells (*25*). In Chinese hamster lung cells treated with crocidolite (origin unspecified), a statistically significant increase in the number of 6-thioguanine resistant cells (indicating forward mutation at the hypoxanthine-guanine phosphoribosyl transferase HGPRT locus) was observed at the one dose (8 μg/mL of fiber) but not at either lower (5 μg/mL) or higher (100 μg/mL) dosages (*25*). In a later study, Huang (*32*) obtained additional evidence that NIEHS chrysotile, crocidolite, and amosite were mutagenic to the HGPRT locus of Chinese hamster lung cells, but these data must be considered suggestive at best since the levels of induced mutation were low and the conclusions strongly dependent on the methods of statistical analysis employed (*32*). Evidence for chromosomal changes in cultured rodent cells following administration of asbestos fibers has been reported in other studies as well (*29, 33*). Thus, it seems clear that asbestos fibers can produce chromosomal aberrations in rodent cells.

With respect to cultured human cells, however, the evidence for chromosomal effects is contradictory. Valerio et al. (*34*) have shown that exposure of freshly isolated lymphocytes (from normal adult males and females) undergo chromosomal changes when treated for 48 and 72 hr with UICC Rhodesian chrysotile A at 10 μg/mL. Increases in numerical alterations after 48 hr and in chromatid and chromosome breaks after 48 hr and 72 hr were statistically significant. At 72 hr, chromatid breaks were the most frequent change noted and the number of numerical alterations were lower than at 48 hr (*34*). In contrast, a comprehensive study by Sincock et al. (*35*) showed that neither cultured human lymphoblasts nor human fibroblasts exhibited chromosomal aberrations when exposed to either UICC crocidolite, SFA chrysotile, or glass fibers (10 μg/mL to 100 μg/mL, 48–72 hr). In contrast, CHO cells exposed under this same regime exhibited numerous chromosomal changes (*35*), a result in concordance with earlier studies (*30*). Both CHO and human cell lines are inhibited to a similar extent with respect to rate of growth by these fiber preparations (*35*).

Sincock (*33*) has reported that UICC crocidolite and SFA chrysotile alter the morphology of cultured mouse 3T3 cells in a manner suggestive of neoplastic transformation; however, quantitative data were not given. Interestingly a preparation of "coarse glass" fibers, previously shown to be inactive as an oncogen in rats, was also ineffective in morphologically transforming these cells (*33*). Likewise, DiPaolo et al. (*36*) have observed a very low level of morphologic transformation in SHE cells by UICC crocidolite, anthophyllite, amosite, and Canadian chrysotile B. However, it would be premature to regard these results as positive, since: (1) the levels of transformants (foci/plate) induced by the fibers alone were very low, (2) the resulting transformed cells were not injected into animals for evaluation of their oncogenicity, and (3) these asbestos fibers have been shown to produce clastogenic effects in SHE cells (*31*). Thus the very low levels of transformants observed by treatment of the fiber alone may be due to the cytogenetic effects.

Thus, while it is clear that asbestos fibers are clastogenic to cultured rodent cells (*25, 29–31, 33*), there is contradictory evidence with respect to human cells (*34, 35*). Since both the cell type and fiber type were different in the two human cell studies in question (*34, 35*) it is, at present, not possible to discern the reason for the discrepancy. In one study, asbestos fibers appeared to produce SCEs in rodent cells (*28*), although two other investigations that reach the opposite conclusion have been reported (*26, 29*). Attempts to demonstrate DNA strand breakage or DNA damage in mammalian cells following asbestos exposure have been negative (*19–21*). The evidence that asbestos fibers *per se* induce either neoplastic transformation (*33, 36*) or gene mutation (*25, 32*) in cultured mammalian cells is weak. Asbestos fibers do not induce either DNA damage (*22*) or back-mutation (*22, 23*) in bacterial cells.

More experimentation may be required before a definitive statement about the genotoxic potential of asbestos mineral fibers can be made; e.g., while a relationship between chromosome instability and cancer has been proposed (*37*), none is proven. While SCE is a cytogenic event that appears to consist of a reciprocal interchange of genetic material between duplicated regions of a chromosome involving a four-strand DNA break, its mechanism is unknown (*28, 38*). Further, the precise relationship between the induction of either mutations (*39, 40*) or chomosomal aberrations (*38*) and SCE is not clear.

Thus, it is concluded that while it is possible that asbestos fibers may act at the stage of tumor initiation (gene toxicity) via a clastogenic event, it may be more reasonable to look at other mechanisms as the explanation for the oncogenic potential of these materials. For example, studies in our laboratory have shown that NIEHS chrysotile, if applied prior to benzo(a)pyrene (BP), increased the level of DNA binding and enhanced the cytotoxic effects of the hydrocarbon to cultured human fibroblasts (*41*). Similar enhancement of BP-DNA binding by preincubation of the

cell cultures with NIEHS "intermediate" chrysotile was also observed in SHE cells (*42*). This latter observation is of interest, since DiPaolo et al. (*36*) have reported a synergism between asbestos fibers and BP with respect to SHE cell transformation. The simultaneous addition of NIEHS chrysotile and BP to normal human fibroblasts did not result in either increased BP metabolism (*41, 43, 44*), altered BP-metabolite profiles, cytotoxicity (*41, 43*), BP-DNA binding levels (*43, 44*), or in substantially altered BP-deoxyribonucleoside adduct profiles (*44*). These studies and others discussed by Mossman (*45*), may indicate that asbestos fibers act as the level of a cocarcinogen or promoter with respect to their oncogenic effects.

This article has been peer and administratively reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Selikoff, I. J., and Hammond, E. C. Asbestos-associated disease in United States shipyards. CA Cancer J. Clin. 28: 87–99 (1978).
2. Cochrane, J. C., and Webster, I. Mesotheliomia in relation to asbestos fiber exposure. S. Afr. Med. J. 54: 279–83 (1978).
3. Wagner, J. C., Gilson, J. C., Berry, G., and Timbrell, V. Epidemiology of asbestos cancers. Brit. Med. Bull. 27: 71–76 (1971).
4. Wagner, J. C., and Berry, G. Mesotheliomas in rats following inoculation with asbestos. Brit J. Cancer 23: 567–81 (1969).
5. Wagner, J. C., Berry, G., and Timbrell, V. Mesotheliomata in rats after inoculation with asbestos and other minerals. Brit. J. Cancer 28: 173–185 (1973).
6. Selikoff, I. J., Churg, J., and Hammond, E. C. The occurrence of asbestosis among insulation workers in the U.S. Ann. N. Y. Acad. Sci. 132: 139-55 (1965).
7. Harrington, J. S., Allison, A. C., and Badami, D. V. Mineral fibers: chemical, physiochemical and biological properties. Adv. Pharmacol. Chemotherap. 12: 291-402 (1975).
8. Mossman, B. T., Light, W. G., and Wei, E. T. Asbestos: Mechanisms of toxicity and carcinogenicity in the respiratory tract. Ann. Rev. Pharm. Toxicol. 23: 595–615 (1983).
9. Mossman, B. T., and Craighead, J. E. Mechanisms of asbestos carcinogenesis. Environ. Res. 25: 169-280 (1981).
10. Berenblum, I. Theoretical and practical aspects of the two stage mechanism of carcinogensis. In: Carcinogens: Identification and Mechanisms of Action (A. C. Griffin and C. R., Shaw, Eds.), Raven Press, New York, 1979, pp. 25-36
11. Lutz, W. K. *In vivo* binding of organic chemicals to DNA as a quantitative indicator in the processes of chemical carcinogenesis. Mutat. Res. 65: 289–356 (1979).
12. deSerres, F. J., and Hollaender, A. (Eds.), Chemical Mutagens: Principles and Methods for Their Detection. Plenum Press, New York, 1980.
13. Butterworth, B. E. (Ed.), Strategies for Short-term Testing for Mutagens/Carcinogens. Chemical Rubber Co., West Palm Beach, FL, 1980.
14. Brown, R. C., Chamberlain, M., Davies, R., Morgan, D. M. L., Pooley, F. D., and Richards, R. J. A comparison of 4 *in vitro* systems applied to 21 dusts. In: The *In Vitro* Effects of Mineral Dusts (R. C. Brown, I. P. Gormley, M. Chamberlain and R. Davies, Eds.), Academic Press, New York, 1980, pp. 47–52.
15. Stanton, M. F., Layard, M., Tegeris, A., Miller, E., May, M., and Kent, E. Carcinogenicity of fibrous glass: pleural response in the rat in relation to fiber dimension. J. Natl. Cancer Inst. 58: 587—603 (1977).
16. Brown, R. C., Chamberlain, M., Griffiths, D. M., and Timbrell, V. The effect of fiber size on the *in vitro* biological activity of three types of amphibole asbestos. Int. J. Cancer 22: 712–727 (1978).
17. Timbrell, V., and Rendall, R. E. G. Preparation of the U.I.C.C. standard reference samples of asbestos. Powder Technol. 5: 279-287 (1971).
18. Campbell, W. J., Higgins, C. W., and Wylie, A. G. Chemical and physical characterization of amosite, chrysotile, crocidolite and non-fibrous tremolite for oral ingestion studies by the National Institute of Environmental Health Sciences. Bur. Mines Report Invest. U.S. DI RI8452 1–63, 1980.
19. Mossman, B. T., Eastman, A., Landesman, J. M., and Bresnick, E. Effects of crocidolite and chrysotile asbestos on cellular uptake and metabolism of benzo(a)pyrene in hamster tracheal epithelial cells. Environ. Health Perspect. 51: 331–335 (1983).
20. Lechner, J. F., Haugen, A., Tokiwa, T., Trump, B. F., and Harris, C. C. Effects of asbestos and carcinogenic metals on cultured human bronchial epithelium. In: Human Carcinogenesis (C. C. Harris, and H. Autrup, Eds.), Academic Press, New York, in press, 1983.
21. Hart, R. W., Fertel, R., Newman, H. A. I., Daniel, F. B., and Blakeslee, J. R. Effects of selected asbestos fibers on cellular and molecular parameters. U.S. Environmental Protection Agency Report No. 600/1–79–21, 1979.
22. Chamberlain, M., and Tarmy, E. M. Asbestos and glass fibers in bacterial mutation tests. Mutat. Res. 43: 159–64 (1977).
23. Light, W. G., and Wei, E. T. Surface charge and a molecular basis for asbestos toxicity. In: The *In Vitro* Effects of Mineral Dusts (R. C. Brown, I. P. Gormley, M. Chamberlain and R. Davies, Eds.), Academic Press New York, 1980, pp. 139–145.
24. Miller, K. The *in vitro* effect of different mineral dusts on the mechanism of phagocytosis. In: The *In Vitro* Effects of Mineral Dusts (R. C. Brown, I. P. Gormley, M. Chamberlain and R. Davies, Eds.), Academic Press, New York, 1980, pp. 91–95.
25. Huang, S. L., Saggioro, D., Michelmann, H., and Malling, H. V. Genetic effects of crocidolite asbestos in Chinese hamster lung cells. Mutat. Res. 57: 225–32 (1978).
26. Chamberlain, M., and Brown, R. C. The cytotoxic effects of asbestos and other mineral dust in tissue culture cell lines. Brit. J. Exptl. Pathol. 59: 183–89 (1978).
27. Kaplan, H., Renier, A., Jaurand, M. C., and Bignon, R. Sister chromatid exchanges in mesothelial cells cultured with chrysotile asbestos. In: The *In Vitro* Effects of Mineral Dusts (R. C. Brown, I. P. Gormley, M. Chamberlain and R. Davies, Eds.), Academic Press, New York, 1980, pp. 251–253.
28. Livingston, G. K., Rom, W. N., and Morris, M. V. Asbestos-induced sister–chromatid exchanges in cultured hamster ovary fibroblast cells. J. Environ. Pathol. Toxicol. 4: 373–382 (1980).

29. Price-Jones, M. J., Grubblings, G., and Chamberlain, M. The genetic effects of crocidolite asbestos: comparison of chromosome abnormalities and sister chromatid exchanges. Mutat. Res. 79: 331–339 (1980).
30. Sincock, A., and Seabright, M. Induction of chromosome changes in Chinese hamster cells by exposure to asbestos fibers. Nature 257: 56–58 (1975).
31. Lavappa, K. S., Fu, M. M., and Epstein, S. S. Cytogenic studies on chrysotile asbestos. Environ. Res. 10: 165–173 (1975).
32. Huang, S. L. Amosite, chrysotile and crocidolite asbestos are mutagenic to Chinese hamster lung cells. Mutat. Res. 68: 265–274 (1979).
33. Sincock, A. Preliminary studies of the *in vitro* cellular effects of asbestos and fine glass dusts. In: The Origins of Human Cancer, Vol B. (H. H. Hiatt, J. D. Watson, and J. A. Winston, Eds.), cold Spring Harbor, 1977, pp. 941–954.
34. Valerio, F., deFerrari, M., Otagio, L., Reptto, E., and Santi, L., Cytogenetic effects of Rodesian chrysotile on human lymphocytes *in vitro*. In: IARC Scientific Publication No. 30, International Agency for Research on Cancer, Lyon, France, 1980, pp. 485–489.
35. Sincock, A. M., Delhanty, J. D. A., and Casey, G. A comparison of the cytogenetic response to asbestos and glass fiber in Chinese hamster and human cell lines. Mutat. Res. 101: 257–68 (1982).
36. DiPaolo, J. A., DeMarinis, A. J., and Doniger, J. Asbestos and Benzo(a)pyrene synergism in the transformation of Syrian hamster embryo cells. J. Environ. Pathol. Toxicol. 5: 535–543 (1982).
37. German, J. Blooms syndrome, II. The prototype of genetic disorders predisposing to chromosome instability and cancer. In: Chromosomes and Cancer. John Wiley and Sons, New York, 1975, pp. 601–617.
38. Perry, P. E. Chemical mutagens and sister chromatid exchange. In: Chemical Mutagens: Principles and Methods for Their Detection (F. J. deSerres, and A. Hollaender, Eds.), Plenum Press, New York, 1980, pp. 1–32.
39. Perry, P. E., and Evans, H. J. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. Nature 258: 121–125 (1975).
40. Carrano, A. V., Thompson, L. H., Lindl, P. A., and Minkler, J. L. Sister chromatid exchanges as an indicator of mutagenesis. Nature 271: 551–553 (1978).
41. Daniel, F. B., Beach, C. A., and Hart, R. W. Asbestos-induced changes in the metabolism of polycyclic aromatic hydrocarbons in human fibroblast cell cultures. In: The *In Vitro* Effects of Mineral Dusts (R. C. Brown, I. P. Gormley, M. Chamberlain and R. Davies, Eds.), Academic Press, New York, 1980, pp. 255–262.
42. Eneanya, D. I., Daniel, F. B., and Hart, R. W. Effect of asbestos (chrysotile intermediate) on the metabolism of benzo(a)pyrene in Syrian hamster embryo cells (abstract). Presented at Am. Soc. Pharmacol. Exptl. Therap. Meeting, Houston, TX, 1979.
43. Hart, R. W., Daniel, F. B., Kindid, O. R., Beach, C. A., Joseph, L. B., and Wells, R. C. Elemental modifications and polycyclic aromatic hydrocarbon metabolism in human fibroblasts. Environ. Health Perspect. 34: 59–68 (1980).
44. Stephens, R. E., Joseph, L. B., Daniel, F. B., Schenck, K. M., Newman, H. A. I., Lipetz, P. D., and Millette, J. R. Interactions of chrysotile and benzopyrene in a human cell culture system. Environ. Health Perspect., in press.
45. Mossman, B. T. *In vitro* approaches for determining mechanisms of toxicity and carcinogenicity by asbestos in the gastrointestinal and respiratory tracts. Environ. Health Perspect. 53: 153–159 (1983).

Environmental Health Perspectives
Vol. 53, pp. 169–176, 1983

Engineering and Operating Approaches for Controlling Asbestos Fibers in Drinking Water

by Gary S. Logsdon*

Techniques are available to minimize the concentration of asbestos fibers in drinking water. Filtration research conducted at locations on Lake Superior and in the Cascade Mountains in Washington has shown that amphibole and chrysotile fibers can be removed by granular media filtration. Removal percentages can exceed 99% when the raw water is coagulated properly and the filtered water turbidity is 0.10 ntu (nephelometric turbidity units) or lower. Filtered water fiber counts below detectable limits of 0.1 to 0.01 × 10^6 fibers/L can be attained. A study by the Metropolitan Water District of Southern California showed that when raw water chrysotile counts ranged from 200 × 10^6 fibers/L to 2000 × 10^6 fibers/L, filtered water fiber counts frequently exceeded 1 × 10^6 fibers/L. Even so, striving to attain a filtered water turbidity of 0.1 ntu resulted in improved fiber removal.

Pilot scale and distribution system research projects have shown that asbestos cement (AC) pipes can be protected from dissolution and leaching effects that can result in release of asbestos fibers into drinking water. Suggested techniques include modifying low pH, low alkalinity waters so they are not aggressive; coating the pipe wall with a chemical precipitate; and applying a cement mortar lining to the pipe wall.

Operation and maintenance practices related to the distribution system, when AC water mains are in service, can influence the fiber count in tapwater. Main flushing can stir up sediment that accumulates in low-flow and dead-end areas, raising the fiber count. If mains are tapped and the cuttings are not flushed away through the tapping machine, but are instead permitted to fall into the water main, the fiber count can be raised.

Introduction

The discovery of asbestos fibers in the drinking water of Duluth, MN, in 1973 caused a high level of interest in the health implications of ingested asbestos. While health effects studies were being planned and carried out, engineering investigations were also undertaken to develop information on effective techniques for removing asbestos fibers from drinking water. At the same time, work was started on evaluation of techniques to control or eliminate the deterioration of asbestos cement (AC) pipe by aggressive waters.

This paper summarizes results of research efforts related to water filtration and to protection or rehabilitation of AC pipe that transmits aggressive water. When asbestos fibers are present in the source water, filtration plants can be designed and operated to remove most of the fibers. When AC pipe is used to transmit aggressive water, formation of a coating on the pipe wall can decrease the extent of deterioration of the pipe and the erosion of fibers from the pipe wall into the water.

Control of Asbestos in Raw Water

Water filtration processes, when operated properly, have been shown to reduce substantially the asbestos fiber concentrations in drinking water. Pilot plant studies in Duluth in 1974 showed that both granular media filtration and diatomaceous earth filtration could produce low turbidity filtered waters with greatly reduced amphibole fiber concentrations (*1*). Treatment of raw water with alum and nonionic polymer was the most effective technique for granular media filtration. Diatomaceous earth (DE) filtration techniques that were most effective involved conditioning the diatomaceous earth filter aid with alum or a

*Drinking Water Research Division, Municipal Environmental Research Laboratory, U.S. Environmental Protection Agency, 26 W. St. Clair St., Cincinnati, OH 45268.

polymer or conditioning the raw water with a polymer. Pressure DE filtration was more effective than vacuum DE filtration.

Filtration plants on Lake Superior were built or remodeled at Duluth, Two Harbors, Beaver Bay, and Silver Bay in Minnesota. The Lakewood Filtration Plant at Duluth has been studied and reported upon extensively (*2–4*). Filtered water generally contained fewer than 0.10 × 10^6 fibers/L and had a turbidity below 0.10 ntu (nephelometric turbidity units). Funds for transmission electron microscope (TEM) analysis of water samples from the other treatment plants were considerably more limited, but some data are available on facilities at Silver Bay and Two Harbors (*5*). These plants use alum as the coagulant and nonionic polymer as a coagulant aid or filter aid. Dual media or mixed media filtration is employed. Plants at Silver Bay and Two Harbors have been able to attain amphibole fiber count reductions of 99% and more (*5*).

Pilot plant studies of granular media filtration were conducted in the Cascade Mountains in 1977–1978. Extensive research was conducted at Seattle's South Fork Tolt Reservoir (*6, 7*). A limited amount of research on asbestos fiber removal was also carried out as a part of a pilot plant study at Everett, Washington (*8*). Chrysotile fiber removal exceeding 99% was demonstrated in both of these projects.

After the Lake Superior and Cascade Mountain studies were completed, pilot plant and full-scale treatment plant operating data were reviewed and summarized (*5*). The relationship between filtered water turbidity and fiber count was scrutinized in a paper by Logsdon et al. (*9*), who concluded that granular media filters should be operated to produce a turbidity of 0.10 ntu or lower if fiber removal is a goal of filter operation. The most important factor involved is properly conditioning the raw water before filtration so that a very low effluent turbidity is produced. Alum and cationic polymers have usually been used for coagulation. Nonionic polymers often are used as filter aids. Control of coagulant dose, to assure thorough and complete particle charge destabilization, is essential, as is careful control of process pH. Filter rates from 2 to 10 gal/min/ft^2 (5 to 24 m/hr) have removed asbestos fibers, but abrupt rate changes must be avoided, particularly if floc is not strong and could pass through the filter as a result of a rate increase. A turbidity of 0.10 ntu was suggested as an appropriate goal for filtered water. Asbestos fiber counts are likely to increase very substantially as filtered water turbidity rises, so increases in turbidity over 0.10 ntu should be interpreted as a signal for corrective action by filter plant operators. Because turbidity is only an indicator of the presence of particulates in water, attaining the lowest possible filtered water turbidity would be the best way to assure that a filtration plant is operating at maximum effectiveness.

A more recent report by McGuire et al. (*10*) questioned whether using 0.10 ntu as a surrogate level for asbestos fibers would be adequate. They reported that filtered water chrysotile concentrations were frequently in the range of 1 × 10^6 fibers/L to 50 × 10^6 fibers/L at three treatment plants of the Metropolitan Water District of Southern California (MWDSC), where raw water fiber counts generally ranged from 200 × 10^6 to 2000 × 10^6 fibers/L. This was observed even when filtered water turbidity in the plant effluent was near or below 0.10 ntu.

The MWDSC study at the treatment plants was divided into two recurring intervals of operation. During standard plant operation, turbidities normally were in the range of 0.20 ntu to 0.35 ntu. In the periods of optimized operation, extra treatment chemicals were used to lower the filtered water turbidity to 0.10 ntu or below as frequently as possible. Probability plots (*10*) showed that

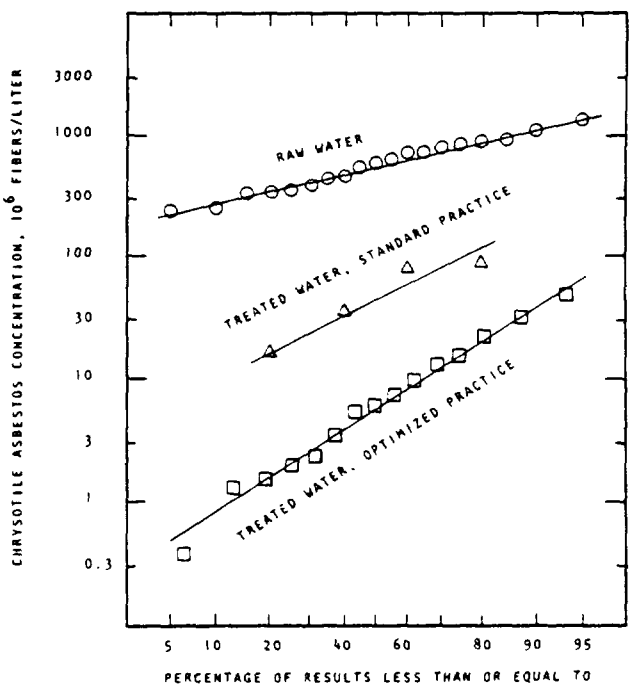


FIGURE 1. Asbestos concentrations in raw and treated water at Weymouth Filtration Plant (data from Metropolitan Water District of Southern California).

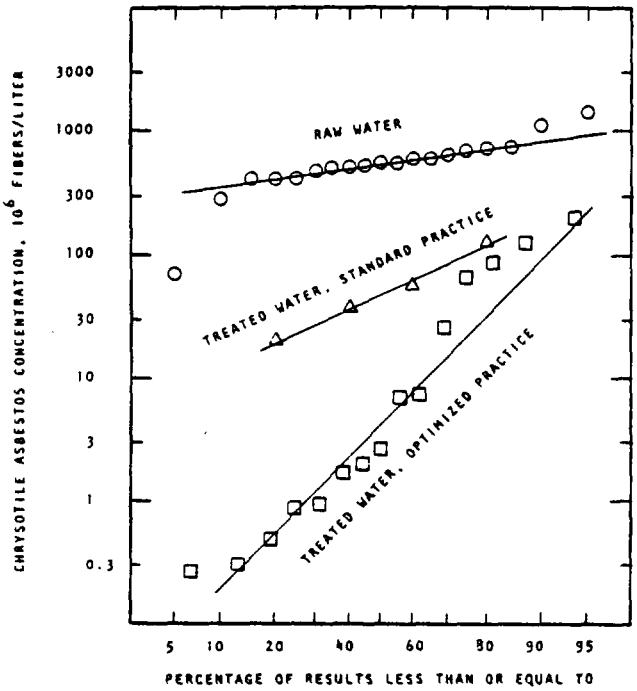


FIGURE 2. Asbestos concentrations in raw and treated water at Diemer Filtration Plant (data from Metropolitan Water District of Southern California).

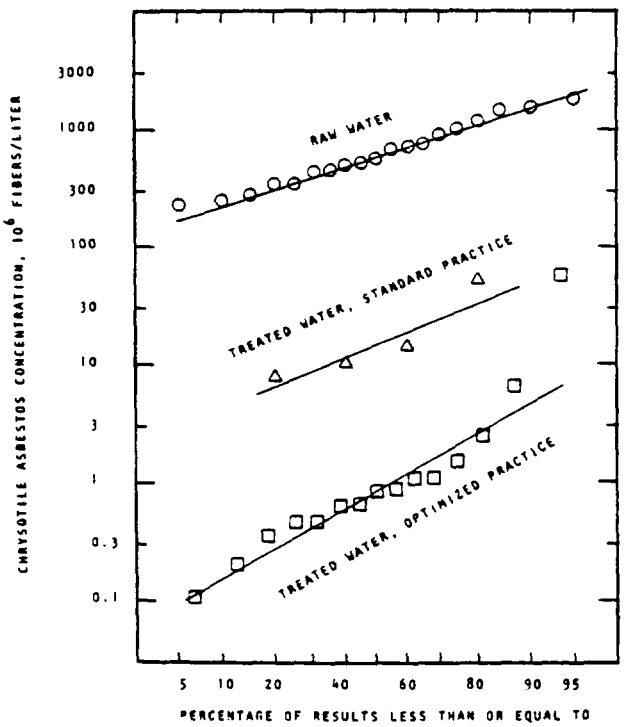


FIGURE 3. Asbestos concentrations in raw and treated water at Mills Filtration Plant (data from Metropolitan Water District of Southern California).

optimized turbidity values were lower, but the comparison of optimized to standard operation was somewhat different for each plant. For the three plants with the highest raw water asbestos concentrations and no filtered water samples with below detectable limit (BDL) values (Weymouth, Diemer, and Mills plants), log-probability plots of filtered water asbestos counts for standardized versus optimized operation are shown in Figures 1–3. The trends of the optimized count data are different for each plant, but for every plant the median value for the optimized operation is considerably below the median for the standardized operation. Even though the fiber counts are usually below 1×10^6 fibers/L or at BDL values, lower fiber counts unquestionably are a result of the optimized operation.

Two important factors may have led to the different conclusions about the value of the 0.10 ntu goal in the Great Lakes—Cascade Mountain studies versus the MWDSC study. First, the concentration of fibers in the raw water source in California was much higher on a continuing basis. Typical raw water amphibole counts at Duluth were 10×10^6 to 200×10^6 fibers/L (*5*), about one-tenth of the California values. In the Seattle Tolt Reservoir study, about four-fifths of the raw water chrysotile counts were in the 2×10^6 to 10×10^6 fibers/L range (*6*). Attaining nearly complete removal of fibers, as indicated by values below the detection limit (BDL), was easier when the raw water fiber count was lower.

A second and important difference is that whereas earlier work focused on the turbidity and fiber concentration of individual filters, in the MWDSC study the treatment plant influent and effluent waters were sampled. This resulted in sampling the average water quality of numerous filters. This distinction is very important because turbidity and fiber count do not have a linear relationship. Fiber counts can increase dramatically with modest increases in turbidity. Data from the Seattle study (*6*) are shown in Table 1. The effect of a turbidity increase in the fiber count can be substantial. For example, at a hypothetical plant with 10 filters producing water with 0.10 ntu and 1.0×10^6 fibers/L, if a turbidity rise in one filter to 0.30 ntu were accompanied by a fiber count increase to 30×10^6 fibers/L, the blended turbidity would average 0.12 ntu, a small increase, but the fiber count would average 3.9×10^6 fibers/L, a large increase. Thus, if all filtered water is piped into a clearwell and the various effluents are blended before turbidity is measured, quality deterioration in a single filter may be overlooked because of the averaging effect of

Table 1. Chrysotile counts associated with turbidity changes in Seattle Tolt Pilot Plant Study.

Run	Hour in run	Turbidity (ntu)	Chrysotile fibers $\times 10^{-6}$, fibers/L[a]
21	6	0.06	0.16 (NSS)
21	7	0.34	12.25
21	8	0.07	0.19
24	6	0.085	0.34
24	7	0.36	6.2
24	13	0.6	0.13
62	3	0.10	0.34
62	9	0.105	0.24
62	16	0.37	2.28
120	12	0.06	0.1 (NSS)
120	13	0.07	<0.01 (BDL)
120	17	0.07	0.02 (NSS)
120	21	0.48	0.28
120	23	1.2	1.25
174 CCM	1	0.070	0.02 (NSS)
174 CCM	1.5	0.21	0.94
174 CMM	2	0.068	<0.01 (BDL)

[a]NSS = not statistically significant; BDL = below detectable limit.

the clearwell. This may have occurred in the MWDSC study.

The differences in raw water quality and sampling technique certainly must be responsible for some of the reported differences in results of various studies, but other factors probably are involved too. Additional research at treatment plants with very high raw water fiber counts is needed to fully explain the results obtained at MWDSC and the apparent shortcomings of the 0.10 ntu quality goal. The Seattle study showed, and the MWDSC indirectly confirmed, that use of a continuous turbidimeter at each filter is needed to enable plant operators to closely monitor and control filtration plant operation.

Control of Asbestos from AC Pipes

Asbestos cement (AC) pipe has captured a larger share of the water distribution system market in recent years. The *American City & County* 1981 Water Main Pipe Survey (*11*) found that the proportion of AC pipe in place was 13.9% in 1980, as compared with 10.8% in 1975; for pipe installed in a single year, it was estimated that 30% of pipe installed in 1974 was AC versus 40% in 1980. The popularity of AC pipe probably results in part from its lower cost in smaller diameters. In sizes up to 12 inches, AC pipe can cost up to 50% less per foot than ductile iron (*11*).

In the early 1970s, concerns about asbestos fibers in drinking water led to investigations of the potential for contribution of asbestos fibers from AC pipes used to carry water. In 1974, a special report on this problem was published by the American Water Works Association (*12*). One of the projects described in the report was an AC pipe loop water recirculation study conducted by Johns-Manville. An EPA project to verify the results of the earlier pipe loop study was begun in 1974 (*13*). When the operation of a pipe loop containing 4-in. and 6-in. AC pipes proved to be extremely difficult, a system of 100-gal. recirculating tanks was developed and used by Buelow et al. (*13*). AC pipe coupons (1.5 × 6 in.) were exposed to the test water in these tanks.

During the same time period, evaluations of AC pipes in field use were conducted at 10 locations (*13*). Four systems with soft, low pH water had deteriorated AC pipe. Problems in one system included clogged water meters, fiber accumulation at strainers on washing machine hoses at a laundromat, and fiber buildup in a kitchen faucet strainer. Thus the field investigations established that aggressive water can attack AC pipe and showed the need for studies of ways to prevent the attack on the pipe or to rehabilitate deteriorated pipe.

Laboratory and field studies have shown that the deposition of a coating may protect AC pipe. Iron coatings were reported by Buelow et al. (*13*) to be protective in a water distribution system and in an experimental pipe loop, but use of iron might result in complaints from rusty or "red" water. Deposition of calcium carbonate layers on AC pipe coupons as well as coating AC pipe coupons with a zinc precipitate was shown to impart protection to the pipe (*13*).

A theoretical explanation of the protection of AC pipe by zinc, iron, and calcium carbonate was given by Schock and Buelow (*14*). They concluded that the Aggressiveness Index (*15*) is not appropriate for predicting fiber release from AC pipe and deterioration of the inner wall of the pipe because the index fails to take into account possible protective constituents such as silica, iron, manganese, and zinc and because the pipe wall is primarily calcium silicate rather than calcium carbonate. They also reported that some coating mechanisms may prevent the release of fibers but not seal the cement pipe matrix from dissolution. In regard to the use of zinc compounds for corrosion control, they concluded that the chloride, sulfate and orthophosphate salts of zinc protected AC pipe by formation of zinc hydroxycarbonate precipitate, but that only zinc orthophosphate

also offered protection for metal pipe in systems containing both AC pipe and lead service lines or plumbing (*14*). AC pipe protection by zinc precipitation is pH dependent. Best results are expected at pH values above 8.0.

Field studies have been performed to evaluate AC pipe protection on a larger scale. At Greenwood, SC (*16*), storage tanks and chemical feed pumps were set up at each of the water treatment plants, and zinc orthophosphate was fed into the system at an average rate of 0.3 mg/L. Two sections of new AC pipe, which were removable for testing, were installed to represent a low-flow and a high-flow water condition. Samples were periodically tested to determine the number of asbestos fibers in the water. The two pipe sections were removed and examined for the amount of zinc deposited on the surface.

Although routine tests, such as pH and alkalinity, showed no significant changes during the study period, asbestos fibers in the water decreased substantially. Electron microscope photographs and energy-dispersive X-ray spectra analyses showed coatings of zinc products on the two pipe samples. Thus, adding zinc orthophosphate under the existing water quality conditions reduced or prevented corrosion of AC pipe.

In a project now underway at Bellevue, Washington, corrosion control strategies have been tested in 100-gal. recirculating tanks, similar to the systems designed and used by Buelow. The tanks at Bellevue are made of polyethylene, rather than stainless steel, minimizing the amount of any kind of metal in the system. The Seattle Water Department, which sells water to Bellevue, has begun to implement a corrosion control program for both the Cedar River water and Tolt River water. Because little or no AC pipe is used in the City of Seattle water distribution system, the objective of Seattle's program is to protect the galvanized pipe and copper pipe in water consumers' plumbing. A corrosion control strategy to protect Bellevue's AC pipe might involve attainment of a water quality different from that of Seattle's or the use of other additivies.

The eight experimental tank tests were started in April 1981 and have been completed. The conditions for each tank are given in Table 2. The zinc chloride corrosion control strategies tested in tanks T5 and T6 were most efficacious in controlling the attack of AC pipe by Tolt water. No substantial difference was observed between the pH 8.0, 0.6 mg/L zinc dose tank (T6) and the pH 8.5, 0.3 mg/L zinc dose tank (T5); both appear to work well. This conclusion is based on water quality sampling during the testing program, asbestos fiber counts, and evaluation of the AC coupons exposed to the test waters. Coupon evaluation included hardness tests, weight loss data, and calcium loss measurements made via scanning electron microscope (SEM). During the next phase of the study, addition of about 0.6 mg/L of zinc at pH 8.0 will be evaluated in a portion of Bellevue's distribution system.

Table 2. Bellevue Pipe Protection Study.

Tank	Source	Condition of treatment
1	Tolt	No corrosion control
2	Cedar	No corrosion control
3	Cedar	Seattle strategy; add lime to pH 8.0
4	Cedar	Seattle strategy; plus lime and soda ash, pH 9.0
5	Cedar	Seattle strategy; plus zinc chloride, 0.3 mg/L Zn, pH 8.5
6	Cedar	Seattle strategy; plus zinc chloride, 0.6 mg/L Zn, pH 8.0
7	Cedar	Seattle strategy; plus sodium metasilicate
8	Cedar	Seattle strategy; plus 0.1-0.3 mg/L Fe (ferric chloride)

Buelow observed that some AC pipe coupons used in the recirculating tank studies had softened when exposed to aggressive water and could be gouged by a fingernail, whereas new AC pipe coupons could not be scratched in this manner (*13*). The hardness of AC coupons was tested at Bellevue to learn if the pipe had been attacked by aggressive water. Two techniques, the Rockwell "L" scale test and the Durometer hardness test have been used. Results were consistent between the two types of test. Leaching of calcium from the AC pipe wall can result in softening of the cement material that bonds the fibers in the pipe. This work is a part of the search for an inexpensive, uncomplicated test to evaluate the condition of the inner pipe wall.

Preliminary hardness testing data indicate reasonable results. Table 3 shows the test data from the testing laboratory. The differences between means were significant at the 0.05 level for both pipe A and pipe B as compared to the new pipe C. These procedures will continue to be used during the course of the study.

Discovery of deteriorated AC pipe in one part of the distribution system of the Town of Weston Water Utility resulted in application for an award of a cooperative agreement for in-place rehabilitation of AC pipe. In 1980, a water main was isolated from the distribution system for the research. Parts of the pipeline were treated by Polly Pigs, bullet-shaped foam swabs forced through the main by hydraulic pressure. Other sections

Table 3. Hardness test results for AC coupons (3/25/81).

	Sample number	Shore D hardness	Rockwell hardness "L" Scale
Pipe exposed to Tolt water	A-1	67	7
	A-2	65	10
	A-3	66	5
	A-4	64	14
Average ± standard deviation		65.5 ± 1.29	9.0 ± 3.92
Used pipe, history unknown	B-1	68	64
	B-2	74	60
	B-3	67	65
	B-4	73	56
Average ± standard deviation		70.5 ± 3.51	61.25 ± 4.11
New pipe	C-1	79	99
	C-2	81	100
	C-3	82	98
	C-4	78	98
Average ± standard deviation		80.0 ± 1.83	98.7 ± 0.96

were cleaned and scraped with metal scrapers that were pulled through the main by the Centriline Department of Raymond International, Inc. In 1981 the entire main (three blocks) was lined with cement mortar.

In this project, Bakelite-mounted specimens of the AC pipe were prepared and studied. The pipe segment was cut and mounted so that the exposed surface of the pipe in the mount had, on one side, the inner pipe wall and, on the other side, the outer pipe wall. This mounting arrangement permitted observation of a cross section of the pipe.

The pipe cross section was studied by an X-ray analysis technique using the scanning electron microscope. Elemental profiles of calcium and silicon concentrations were produced. In new AC pipe, the calcium and silicon concentrations are constant from the pipe inner wall through the pipe to the outer wall. In used pieces of pipe from Weston, however, the silicon concentration was constant, but the calcium concentration was lower near the inner wall when compared to other locations deeper within the pipe wall. The depth of the pipe showing lower calcium concentrations ranged from 2.1 mm to 6.5 mm. Analysis of AC pipe cross sections from four other cities showed calcium losses to depths ranging from 0.8 mm to 3.6 mm. This technique produces a quantitative measure of pipe deterioration.

Results of analysis for asbestos fibers in water circulated in the Weston test mains suggest that although scraping or pigging AC mains can remove the softened portion of the pipe wall, such procedures greatly increase the fiber count of water transmitted in the pipes. Making firm conclusions about the effect of cement mortar lining as a barrier to passage of asbestos fibers from AC pipe into drinking water is not possible because of the poor precision of the asbestos counting procedure when fiber concentrations are low. Nevertheless, a mortar lining would be expected to be a positive barrier to passage of fibers from the pipe wall into drinking water.

Support for the efficacy of the mortar lining as a barrier to asbestos fibers comes from another mortar lining project. Test sections of vinyl-lined AC pipes were lined with cement mortar in the Town of Ashland, Massachusetts, in order to reduce or prevent the leaching of 1,1,2,2-tetrachloroethylene (TCE) into drinking water (*17*). Two mortar-lined sections of AC pipe were compared for a 2-month period with two control sections of vinyl-lined AC pipe. In the test section from which the vinyl liner was removed before the mortar lining was applied, TCE concentrations in the static test water were reduced by 96.2–99.5% as compared to the controls. TCE reductions ranging from 98.2–99.4% were observed in the test pipe in which the cement mortar lining had been placed directly over the vinyl liner. TCE concentrations in the mortar-lined pipes ranged from 25 to 1169 μg/L, while concentrations in the control pipes ranged from 2800 to 74,800 μg/L. Because of the precision with which TCE can be measured in water, much greater confidence can be placed in the Ashland, Massachusetts, TCE data than in the Weston, WI, asbestos data. The Ashland results indicate that because a cement mortar lining can greatly reduce leaching of organic molecules into water, the lining should be a positive barrier to the passage of fibers into water from AC pipe.

Distribution system operation and maintenance procedures can have a substantial impact on the fiber count of drinking water transmitted by AC pipe. According to Buelow et al. (*13*), when AC pipe under pressure is drilled and tapped, if the cuttings are not flushed out through the tapping machine, they can drop into the pipe and cause a major release of fibers. This problem can be avoided by using tapping machines equipped with flushing devices.

High water flows in the distribution system can also cause high fiber counts. High flows caused by flushing or firefighting can stir up sediment in AC mains. If this happens in low-flow areas, fiber counts can rise to 10 or even 100 times the typical values found when flow conditions are normal. In

one Wisconsin system (*18*), flushing an AC water main for 1 min resulted in a concentration of about 10 billion fibers/L in a sample from a fire hydrant. Fiber concentrations in undisturbed household tap samples were about 5–7 million fibers/L. Main flushing, when it is done, should be carried out for a long enough time to thoroughly remove debris from water mains. This has been investigated at Weston, but further study may be needed before definitive recommendations can be made.

Summary

Water filtration for asbestos fiber removal has been studied extensively in Duluth, MN, and in other Minnesota communities located on Lake Superior; in the Cascade Mountains in Washington; and in southern California. Diatomaceous earth filters and granular media filters have been shown to be capable of removing asbestos fibers from source waters.

Corrosion control techniques and water distribution system conditions have been evaluated to show what approaches might be taken to prevent the deterioration of AC pipe carrying aggressive water. Studies have been done at the Drinking Water Research Division's laboratory in Cincinnati, in South Carolina, in Washington, and elsewhere. Each project focused on the effects of various kinds of coatings that may form on pipe walls. A Wisconsin study was also conducted to evaluate what may be the most durable of all coatings, a cement mortar lining.

Water distribution system research revealed that utility operating practices can influence asbestos fiber concentration in drinking water. Drilling and tapping can introduce asbestos-containing debris into the distribution system unless pipe fragments are flushed out under pressure during the tapping process. After asbestos-containing debris has accumulated in the low-flow or dead-end areas of distribution systems, flushing such water mains can cause unusually high fiber counts.

Although the health effects issues are not completely resolved, EPA has recommended that asbestos exposure be minimized when possible (*19*). The techniques used to minimize asbestos exposure may also bring about other water quality improvements. More effective water filtration has greater potential for removal of viruses, Giardia cysts, and bacteria as well as fibers. An active corrosion control effort may help to prevent other corrosion problems in metal pipes. Better distribution system operation and maintenance practices will decrease the amount of debris passing to the taps of water consumers. Attaining these improvements would be consistent with good water works practice.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Black & Veatch, Consulting Engineers. Direct Filtration of Lake Superior Water for Asbestiform Fiber Removal: Project Report and Appendices A through I. EPA-670/2-75-050a through EPA-670/2-75-050g.
2. Patton, J. L. Unusual water treatment plant licks asbestos fiber problem. Water Wastes Eng. 14 (11): 41–44, 50 (1977).
3. Peterson, D. L. The Duluth experience: asbestos, water and the public. J. Am. Water Works Assoc. 70(1): 24–28 (1978).
4. Logsdon, G. S., Schleppenbach, F. X., and Zaudtke, T. M. Filtration works out asbestos fibers. Water Sewage Works 126 (10): 44–46 (1979).
5. Logsdon, G. S. Water Filtration for Asbestos Fiber Removal. U.S. Environmental Protection Agency, Cincinnati, 1979, EPA-600/2-79-206.
6. Kirmeyer, G. J. Seattle Tolt Water Supply Asbestiform Removal Study (Project Report and two Appendix Reports). U.S. Environmental Protection Agency, Cincinnati, 1979, EPA-600/2-79-125, EPA-600/2-79-126, and EPA-600/2-79-153.
7. Kirmeyer, G. J., Logsdon, G. S., Courchene, J. E., and Jones, R. R. Removal of naturally occurring asbestos fibers from Seattle's Cascade Mountain water source. In: Proceedings of the American Water Works Association's Annual Conference, San Francisco, 1979.
8. Watkins, J., Ryder, R. A., and Persich, W. A. Investigation of turbidity, asbestos fibers and particle counting techniques as indices of treatability of a Cascade Mountain water source. In: Proceedings of the American Water Works Association's Annual Conference, Atlantic City, NJ, Part 2, 1978, p. 33–3 ff.
9. Logsdon, G. S., Symons, J. M., and Sorg, T. J. Monitoring water filters for asbestos removal. J. Environ. Eng. Div., Proc. A.S.C.E. (107) EE6: 1297–1315 (1981).
10. McGuire, M. J., Bowers, A. E., and Bowers, D. A. Optimization of large-scale water treatment plants for asbestos fiber removals. In: Proceedings of the American Water Works Association's Annual Conference, Miami Beach, FL, Part 2, 1982, pp. 1215–1244.
11. Anonymous. Cost, not material, shifting pipe choice. Am. City & County 96 (6): 41–44 (1981).
12. Wright, G. W., Kuschner, M., Lee, R., Robeck, G. G., Rossum, J. R., Schneiderman, M. A., and Taylor, E. W. Does the use of asbestos-cement pipe for potable water systems constitute a health hazard? (Committee Report). J. Am. Water Works Assoc. 68 (9) Part 2: 1–22 (1974).
13. Buelow, R. W., Millette, J. R., McFarren, E. F., and Symons, J. M. The behavior of asbestos-cement pipe under various water quality conditions: a progress report, part

1—experimental results. J. Am. Water Works Assoc. 72 (2): 91–102 (1980).
14. Schock, M. R., and Buelow, R. W. The behavior of asbestos-cement pipe under various water quality conditions: part 2, theoretical considerations. J. Am. Water Works Assoc. 73 (12): 636–651 (1981).
15. AWWA Standard for Asbestos-Cement Pressure Pipe, 4 in. Through 24 in., for Water and Other Liquids. AWWA C400-77, Rev. of C400-75, Am. Water Works Assoc., Denver, Colorado, 1977.
16. Grubb, C. E. Field Test of Corrosion Control to Protect Asbestos-Cement Pipe. NTIS Report PB 81-152 878, National Technical Information Service, Springfield, VA (1981).
17. Yuskus, L. R. Tetrachloroethylene (TCE) concentration reduction in vinyl-lined asbestos cement water mains through the application of cement mortar linings. J. New England Water Works Assoc. 97(2): 90–113 (June 1983).
18. Millette, J. R., Pansing, M. F., and Boone, R. L. Asbestos-cement materials used in water supply. Water/Engineering Management 127 (3): 48, 51, 60, 97 (1981).
19. Federal Register 45 (168): 57350 (Wednesday, Aug. 27, 1980).

Environmental Health Perspectives
Vol. 53, pp. 177–180, 1983

Asbestos in Drinking Water: A Canadian View

by P. Toft* and M. E. Meek*

For several years now, public health professionals have been faced with evaluating the potential hazards associated with the ingestion of asbestos in food and drinking water. In Canada, this is a subject of particular concern, because of the widespread occurrence of chrysotile asbestos in drinking water supplies.

The results of available Canadian monitoring and epidemiologic studies of asbestos in drinking water are reviewed and discussed in light of other published work. It is concluded that the risk to health associated with the ingestion of asbestos, at the levels found in municipal drinking water supplies, is so small that it cannot be detected by currently available epidemiologic techniques.

Asbestos is a commercially important mineral in Canada, which is second only to the Soviet Union in world production. Although the mineral also occurs in Newfoundland, British Columbia, Ontario, and the Yukon, mining is concentrated chiefly in Quebec. Asbestos was first mined in Canada in 1878, and production today is about 1,500,000 metric tons/yr (*1*). About 95% of the asbestos produced in Canada is exported, largely to the United States and Europe.

The hazards to health that can result from inhalation of asbestos dust in the occupational environment have long been recognized, and, as a result, most countries today have an occupational standard of 2 fibers/mL or better. However, asbestos is also a ubiquitous environmental contaminant because of its extensive industrial use and the dissemination of fibers from natural sources. Asbestos has been detected in the air of many of our cities and in our food and drinking water. Therefore, for several years we have been faced with the question of whether the ingestion of asbestos presents a public health hazard. In this workshop we have been fortunate to learn about the results of some very interesting research carried out by U.S. and other scientists in an attempt to answer this question. I would like to review some of the Canadian contributions to this field.

The presence of asbestos fibers in drinking water was first reported in 1971 by Cunningham and Pontefract in a study conducted at Health and Welfare Canada (*2*). In addition to determining the asbestos content of a variety of alcoholic beverages, Cunningham and Pontefract analyzed the drinking water supplies of eight municipalities. Although their methodology could be criticized when compared with today's techniques, these investigators nevertheless provided the first information on asbestos concentrations in public drinking water supplies.

Six years later, in 1977, Wigle (also from Health and Welfare Canada) studied cancer mortality for the period 1964–73 in 22 municipalities in Quebec (*3*). Two of the municipalities, Asbestos and Thetford Mines, were known to have high concentrations of asbestos in their drinking water supplies. In the Cunningham and Pontefract study, the levels measured were approximately 200 million fibers/L. Asbestos has been mined in these two communities since 1878 and 1882, respectively, so it can be assumed that the population has ingested drinking water containing substantial concentrations of asbestos for many years. Other communities were selected as likely to have high asbestos concentrations in their water supplies because either they were located immediately adjacent to asbestos-containing deposits or they obtained their drinking water supplies from rivers that drain the regions of such deposits. Community water supplies with probable low concentrations were selected, based on their location being remote from peridotite deposits.

*Monitoring and Criteria Division, Bureau of Chemical Hazards, Environmental Health Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada K1A 0L2.

Cancer mortality data and population data were obtained from the Health Division of Statistics Canada. Significantly high cancer mortality for all cancer sites combined was observed for men in both Asbestos and Thetford Mines (observed = 195, expected = 158.4), but not for women (observed = 127, expected = 138.2). Mortality due to cancer in all gastrointestinal sites was not elevated in men or women in these two high-exposure communities. A slight excess of mortality from stomach cancer was observed in men only; similarly, a small excess in mortality from cancer of the pancreas was observed in women only. However, substantial excess lung cancer mortality was noted among men (observed = 78, expected = 46.8). Since a large proportion of the labor force in both Thetford Mines and Asbestos is employed in asbestos mining and milling, it seemed likely that the occupational exposures would account for the excess mortality among males due to cancers of the stomach and lungs. The authors therefore concluded that the study "did not reveal excess cancer mortality that could be related to the presence of asbestos fibres in drinking water supplies."

In 1977, a national survey of asbestos concentrations in drinking water was conducted in Canada (*4*). Water samples were taken from 71 locations across the country, and the water supplies of more than half of the population were sampled. Most of the sampling was conducted in large cities, but the water supplies of small towns in the mining areas were also examined. Samples of raw, treated, and distributed water were collected from all locations. This type of sampling scheme was used to evaluate the impact of the water treatment and distribution system on fiber concentration.

The samples were analyzed for asbestos content using the U.S. EPA interim procedure. This involved filtration through a Nuclepore filter, which was then carbon-coated under vacuum. The filter material was then dissolved away, leaving a carbon replica of the filter surface with the particles embedded in it. To obtain a good representation of the deposit over the whole area of the filter, three grids were prepared from different parts of each filter. The fibers present in 10 grid openings selected from all specimen grids were counted on the transmission electron microscope. By using this procedure, a precision of ± 30% was obtained in most cases, the precision of the fiber count being limited largely by the counting statistics. Morphology was used as the primary criterion for identifying fibers as either amphibole or chrysotile. Supporting evidence was provided by selected area electron diffraction and energy dispersive X-ray analysis.

The survey revealed that amphibole asbestos is not a significant contaminant of Canadian drinking water supplies. Only 7% of 336 samples showed detectable levels of amphibole fibers, and they were generally associated with high chrysotile levels.

Chrysotile asbestos was identified as the major asbestos type present in drinking water. At the time of the survey, 5% of the population received water with asbestos concentrations greater than 10 million fibers/L. About 0.6% received water with more than 100 million fibers/L. The highest result for a tapwater sample in this survey was 1,800 million fibers/L, which was recorded for Baie Verte in Newfoundland. In this particular case, the provincial government initiated measures to reduce the levels. The authors also obtained considerable information about the nature of the fibers in these samples. In general, the median lengths observed were between 0.5 and 0.8 μm. There was also an indication in at least one and perhaps two cities that erosion of asbestos-cement pipe was contributing to the asbestos content of drinking water.

The relationship betwen the mortality rates for a variety of diseases and the asbestos levels in the drinking water of the surveyed communities was then examined (*1*). The 71 cities surveyed were divided into two groups: those with asbestos concentrations in drinking water greater than 100 million fibers/L and those with less than 5 million fibers/L. Those cities with intermediate concentrations and those with small populations (less than 10,000) were excluded from the study. There were two cities (Sherbrooke and Thetford Mines) in the relatively high group and 52 cities in the low group.

The death rates from various causes in these two groups were compared in the following way. The localities were defined according to the 1976 census geographic boundaries. All deaths among usual residents of the localities were retrieved from the computerized national mortality files. The population at risk was estimated, using data from the censuses of 1966, 1971, and 1976, and adjusted to 1976 geographic boundaries. Person-years at risk by sex and age during the period from 1966 to 1976 were calculated for each locality. Age-standarized mortality rates (ASMRs) were calculated for the age range of 25 to 69. This range was selected for comparison because the reliability of the certified cause of death in this range is greater than that for older persons. The results showed that the all-cause and all-cancer

mortality rates for males in Thetford Mines were significantly higher than those for Sherbrooke or the 52 comparison localities, but the rates for females were similar in all three regions. Mortality rates for nonneoplastic diseases of the respiratory system were significantly high for males in Sherbrooke and Thetford Mines; the rate for females in Thetford Mines was significantly low.

Particular attention was paid to the incidence of cancers of the gastrointestinal (GI) tract. The only high ASMR of statistical significance among the GI cancers was that for stomach cancer in males in Thetford Mines; the mortality rate of 38.7 was more than double the rate in the comparison group, which was 16.8. No significant difference was observed for other GI cancers between the "high" and "low" groups. The only other statistically significant result was the high mortality rate for lung cancer among males in Thetford Mines.

The authors concluded that, among males in Thetford Mines, the high mortality rates due to lung cancer and stomach cancer were probably caused by occupational exposure. Thetford Mines is one of the oldest asbestos mining communities in Canada.

In this context, it is particularly interesting to examine in detail the data for Sherbrooke. For many years, the source of the drinking water supply for Sherbrooke has been a lake that is contaminated by natural serpentine deposits. The water is not filtered during treatment. In the 1977 survey, the concentration of asbestos in drinking water samples taken from the distribution system was found to be 153 million fibers/L. The population is relatively large (93,000 in 1971), and, most importantly, there is no asbestos mining acitivity in the immediate region. Age-adjusted mortality rates for Sherbrooke were, therefore, compared with those for seven municipalities with low asbestos concentrations in their drinking water supplies. These municipalities were matched for water source, chlorination status, and population size (50,000–100,000). For both men and women, mortality rates for all GI cancers combined were, in fact, found to be lower in Sherbrooke than in comparison cities. Only minor, statistically insignificant differences were observed for cancers of specific GI sites and indeed, for all other sites.

The Canadian studies thus provide no consistent, convincing evidence of increased cancer risks attributable to the ingestion of drinking water contaminated by asbestos, even though the observed asbestos concentrations were relatively high in several communities.

Although several ecologic epidemiologic studies have been conducted to date, evidence of increased cancer incidence has been associated with the ingestion of asbestos in drinking water in only one investigation, i.e., the one conducted in the San Francisco Bay Area (*6*). Although this study was carefully designed, several factors complicate the interpretation of the results of the San Francisco Bay study and indeed of ecologic epidemiologic studies generally. Several of these confounding factors have been described in this workshop. It is reassuring that the association observed in the California study has not been confirmed in the Puget Sound case-control study, which included assessment of exposures and outcomes for individuals rather than populations.

Based on a review of the toxicologic studies on ingested asbestos, we believe that there is no conclusive evidence that ingested asbestos is carcinogenic or cocarcinogenic in animal species. The early studies were inconclusive due to shortcomings in study design. In the more complete studies conducted more recently, the incidence of GI tumors in the asbestos-fed animals has not been significantly greater than that in the control groups. These results were also confirmed by the extensive National Toxicology Program study described at this workshop. There is also no evidence of a dose-response relationship in any of the studies.

The absence of definitive evidence of carcinogenicity in the epidemiologic and toxicologic studies may be due to GI-induced changes that reduce the carcinogenic potential of the fibers. Another possibility is that the GI mucosa presents an effective barrier to the penetration of asbestos fibers. Data concerning the penetration of asbestos fibers into and through the GI tract are contradictory, and the interpretation of the results of available studies is complicated by factors such as the insensitivity of available analytical techniques and the possibility of contamination during sample preparation. It seems unlikely, however, that there is widespread penetration of ingested asbestos fibers into and through the GI tract.

On the basis of the available data, we conclude that the risk of disease associated with the ingestion of asbestos at levels found in drinking water supplies is probably extremely small.

The work described in this paper was sponsored by the Department of National Health and Welfare, Ottawa, Ontario, Canada. The viewpoints expressed do not necessarily reflect the view of the U.S. Environmental Protection Agency.

REFERENCES

1. Toft, P., Wigle, D. T., Meranger, J. C., and Mao, Y. Asbestos in drinking water in Canada. Sci. Total Environ. 18: 77–89 (1981).
2. Cunningham, H. M., and Pontefract, R. D. Asbestos fibres in beverages and drinking water. Nature 232: 332–333 (1971)
3. Wigle, D. T. Cancer mortality in relation to asbestos in municipal water supplies. Arch. Environ. Health 32: 185–190 (1977).
4. Chatfield, E. J., and Dillon, M. J. A National Survey for Asbestos Fibres in Canadian Drinking Water Supplies. Department of National Health and Welfare, 1979, 79-EHD-34, Ottawa, Canada.
5. Wigle, D. T., Mao, Y., Toft, P., and Meranger, J. C. Cancer mortality and drinking water quality in selected Canadian municipalites. In: Proceedings of the International Joint Commission Workshop on the Health Effects of Great Lakes Water Quality, Windsor, 1981.
6. Conforti, P. M., Kanarek, M. S., Jackson, L. A., Cooper, R. C., and Murchio, R. C. Asbestos in drinking water and cancer in the San Francisco Bay Area: 1969-1974 incidence. J. Chron. Dis. 34: 211–224 (1981).

Environmental Health Perspectives
Vol. 53, pp. 181-183, 1983

Commentary

Asbestos in Drinking Water: A Status Report

by Joseph A. Cotruvo*

The conference is briefly reviewed in the light of its impact on future regulatory decisions regarding the possible control of asbestos fiber in drinking water.

The results of animal feeding studies indicate that asbestos fails to demonstrate toxicity in whole-animal lifetime exposures. The epidemiologic evidence of risk from ingestion of water containing asbestos fibers is not convincing, and in view of the lack of confirmation by animal studies, the existence of a risk has not been proven; however occupational gastrointestinal cancer may indicate ingestion risk.

Whether or not there is a risk from asbestos in drinking water, however, common sense tells us to deal with an undesirable situation by employing means that are commonly and economically available. Well-known methods can minimize the presence of asbestos fibers in finished drinking water. In the case of natural fiber in raw water, standard or augmented filtration practices are extremely effective. If the source of asbestos fiber is asbestos-cement pipe that is being attacked by corrosive water, then, there is more than sufficient economic reason to correct the corrosivity of the water.

I have been asked to make some observations about the direction of activities in the United States and the Environmental Protection Agency in dealing with asbestos contamination of drinking water. I will also comment on this conference as it relates to those activities. My discussion will be confined to "where do we go from here" in the context of the Safe Drinking Water Act, which controls decision-making regulatory activities affecting drinking water contaminants.

The debate on asbestos and the human risk from ingestion of asbestos fibers has been going on since at least 1971 (*1–3*). One of the earliest questions to be asked was, in the light of the clear carcinogenesis of inhaled asbestos, what is EPA's regulatory posture related to ingested asbestos from drinking water? Our response, at that time, was that we did not feel that there were sufficient data on which to make a judgment on the risk. We recommended, however, that where asbestos fibers were found in drinking water, some of the many available means for minimizing fiber concentrations should be utilized to avoid unnecessary exposure.

The issue has ripened considerably since then. Virtually all of the data, including the results of research on epidemiology and animal feeding studies, transport phenomena, and *in vitro* studies, have been summarized in this program. Very few other issues have had the volume and intensity of study and resultant information that has been presented here, and there is no question that the importance of the issue warranted that m[illegible] attention. Based on all of the information, an[illegible] the context of the Safe Drinking Water Act, we have to answer a few questions. First, is there a risk associated with the ingestion of asbestos fibers in drinking water? Second, if so, what is the magnitude of that risk? Finally, if it turns out that the risk, if any, is of sufficient magnitude, what kind of judgment should EPA make in a regulatory context?

My understanding of the animal and human epidemiology data, based on the discussions of this meeting, is that there is a substantial question as to whether there is a risk at all from small amounts of asbestos fibers in drinking water. On the other hand, if there is a risk, the magnitude of that risk must be quite small at fiber levels typically found in drinking water or perhaps even at atypical levels, such as the 100-million-plus fiber per liter concentrations.

There have been several analyses that have attempted to estimate the upper limit of that risk, and one of these was performed in 1980 (*4*). Extrapolations were made from the occupational

*Criteria & Standards Division, Office of Drinking Water, Environmental Protection Agency, 401 M Street, SW, Washington, DC 20460.

inhalation data to project gastrointestinal intake, and several fairly tenuous conservative assumptions were included in that analysis. One of the estimates indicated an approximate upper excess risk of one in 100,000 per lifetime for gastrointestinal cancer at 300,000 fibers/L for 2 L water ingested per day for 70 years. The available data indicate that substantial populations are exposed to this concentration of fibers in drinking water in the United States and Canada; approximately 5 to 10% of the population in this country may be consuming asbestos-containing water at that level. The fundamental question is, though, does this constitute a significant risk? If it is a real risk, then what is the evidence that indicates the existence of that risk and what is the best quantitative estimate of the risk?

Under the provisions of the Safe Drinking Water Act, the choices EPA has are: (a) to regulate by providing some sort of legal limit: (b) not to regulate, but to provide guidance (which often is well received and followed); (c) or to conclude that there are not sufficient data on which to base regulations or provide guidance. If EPA were to regulate, the options would be to establish a numerical limit, called a maximum contaminant level (MCL) expressed in terms of a fiber count, or mass, or to require the application of one or more specific treatment processes known to be capable of reducing the fiber concentration. Either of these approaches would establish a ceiling on the amount of asbestos that could be present in drinking water in a given situation. The decision on alternative approaches, by law, must be based on the availability and feasibility of analytical methodology. The previous discussions have indicated that the analytical technology, although existing in certain sophisticated laboratory settings, is probably not widely available to public water systems, so perhaps there are other approaches to be taken if a limit is necessary.

The results of animal feeding studies as presented, including the National Toxicology Program study, indicate essentially that no toxicity was demonstrated in whole animal lifetime exposures (*5,6*). In the epidemiology studies, it appeared that in the one ecological study where there was some apparent correlation between asbestos fiber concentration and cancer risk, there were some confounding factors that had not been, or could not be, considered in that particular case, thus rendering the correlations questionable (*7,8*). Reanalysis of the data (*9*) seemed to produce anomalous results when San Francisco (city) populations were differentiated from others. A case-control study (*10*), using a smaller sample population, in the Pacific Northwest, apparently did not detect the existence of a measurable risk due to exposure to asbestos fibers at a level much higher than that found in the ecological study in the Bay Area of California. It can be said, then, that the epidemiologic evidence of risk from ingestion of water containing asbestos fiber is not convincing, and that, in view of the lack of confirmation by animal studies, the existence of a risk has not been satisfactorily demonstrated. There remains, however, the vexing question of the gastrointestinal cancer risk reported from occupational exposure studies (*11*).

There apparently is some migration of asbestos fibers *in vivo*. There also apparently are chemical transformations that occur in the gastrointestinal tract—transformations that probably do not occur in the respiratory area. This might explain apparent differences in the toxicology of asbestos depending on the route of exposure. The *in vitro* mutagenicity studies appear to be for the most part negative, but there does appear to be some element of cocarcinogenicity of asbestos with some polycyclic aromatic hydrocarbons. This situation is one that might call for additional study.

In order to make a decision on the regulation of absestos in drinking water, EPA must consider all of the available information, not only the credibility and magnitude of the risk, but also the analytical science, the treatment technology and the cost of control. Within the context of the Safe Drinking Water Act, the mechanism for making the decision is operating right now.

An Advance Notice of Proposed Rulemaking (ANPRM) will appear in the *Federal Register*. This ANPRM relates to EPA's total revision of the existing National Primary Drinking Water Regulations and the possible inclusion of additional contaminants in Revised Primary Regulations; asbestos is one of several candidates to be mentioned in the ANPRM. There will then be several workshops, a public meeting, and other opportunities to debate the issue. The ultimate decision goes beyond science and calculations—it becomes a function of a social judgment in the context of the Safe Drinking Water Act in terms of the appropriate decision based upon a weighing of all of the available information.

The next step in the regulatory development process is to decide on whether to propose a regulation. That decision will probably be made in early 1984. In the meantime, in addition to the proceedings of the workshops, advice will be available from the Safe Drinking Water Committee of the National Academy of Sciences which is now reviewing the matter for the Office of Drink-

ing Water. While sufficient data on which to base a decision may not have been available in the past, it appears that we are now at a point where there is adequate information along with the appropriate mechanism for reaching a decision in a reasonable period of time.

The issue of asbestos in drinking water falls into the same category as most of the environmental contamination issues. We never have the absolute, unequivocal yes/no answer, but of all the issues that we have examined in the past, the asbestos research has probably produced more hard data than the others. Therefore, I believe we will be able to make a good decision based on the current facts.

Water that is corrosive toward any element in a public water system is likely to be unacceptable for both economic and public health reasons. Apart from the forthcoming regulatory decision and regardless of whether there is a demonstrated risk from asbestos in drinking water, common sense tells us to deal with an undesirable situation by employing means that are commonly available, and to do so economically. Well-known methods are available to minimize the presence of asbestos fibers in finished drinking water. In the case of natural fiber in raw water, standard or augmented filtration practices are extremely effective. There are many good reasons why surface water should be filtered, and the presence of asbestos is just one more of them. If the source of asbestos fiber is asbestos-cement pipe that is being attacked by corrosive water, then there is more than sufficient economic reason to correct the excessive corrosive action of the water on the pipe.

Additional extensive research is not likely to shed much more light on the question of the risks of asbestos in drinking water. It is EPA's responsibility to make a decision using the data that we now have; it is the water industry's responsibility to provide drinking water for its customers that is as safe and wholesome and as free from adulterants as can be economically achieved using filtration or corrosion control techniques that are readily available.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Cunningham, H. M., and Pontefract, R. D. Asbestos fibers in beverages and drinking water. Nature 232: 332–333 (1971).
2. Cook, P. M., Glass, G. E., and Tucker, J. H. Asbestos amphibole minerals: detection and measurement of high concentrations in municipal water supplies. Science 185: 853–855 (1974).
3. Nicholson, W. J. Analysis of amphibole asbestiform fibers in municipal water supplies. Environ. Health Perspect. 9: 165–172 (1974).
4. Ambient Water Quality Criteria for Asbestos. U.S. Environmental Protection Agency, Washington, DC, 1980, EPA 440/5–80–022.
5. NTP. Carcinogenesis Bioassay of Amosite Asbestos in Syrian Golden Hamsters. Draft Report. National Toxicology Program, Research Triangle Park, NC, 1981.
6. NTP. Carcinogenesis Bioassay of Chrysotile Asbestos in Syrian Golden Hamsters. Draft Report. National Toxicology Program, Research Triangle Park, NC, 1981.
7. Kanarek, M. S., Conforti, P. M., Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer incidence in the San Francisco Bay Area. Am. J. Epidemiol. 112: 54–72 (1980).
8. Conforti, P. M., Kanarek, M. S., Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer in the San Francisco Bay Area. J. Chron. Di[illegible]: 211–24 (1981).
9. Tarter, M. E., Cooper, R. C., and Freeman, W. R. A graphical analysis of the interrelationships between waterborne asbestos, digestive systems, cancer and population density. Environ. Health Perspect. 53: 79–90 (1983).
10. Polissar, L., Severson, R. K., and Boatman, E. S. Cancer risk from asbestos in drinking water: summary of a case-control study in western Washington. Environ. Health Perspect. 53: 57–60 (1983).
11. Hammond, E. C., Selikoff, I. J., and Churg, J. Neoplasia among insulation wokers in the United States with special reference to intra-abdominal neoplasia. Ann. N. Y. Acad. Sci. 132: 519–525 (1965).

Environmental Health Perspectives
Vol. 53, pp. 185–187, 1983

Commentary

Additional Thoughts on the Review of Epidemiologic Studies Related to Ingested Asbestos

by Gary M. Marsh*

Following my presentation (*1*), a number of very interesting questions were raised. I am happy to be able to discuss some of these issues in this commentary. One question dealt with the fact that the concentration of fibers in Connecticut (*2,3*) was very low. Could any positive result really be expected? Since a dose-response relationship between ingested asbestos and related cancers has not been established, it is not possible to characterize any exposure level as insufficient for increasing cancer incidence or mortality above background levels. In fact, this basic uncertainty has probably provided much of the motivating force behind the ecological studies that have been conducted to date. If one subscribes, however, to the notion of the "existence" of a positive dose-response relationship between ingested asbestos and cancer, then, of course, the probability of detecting an elevated risk would be enhanced by studying populations that are exposed to higher levels of waterborne asbestos.

Another question concerned the use of multiple regression in the Connecticut study: How well did the data fit the normal assumptions for multiple regression? The questioner felt that a perfect fit cannot be expected, and that these kinds of data are usually fraught with outliers. He also suggested that the variance of observations is also probably not constant. I have found that it is not uncommon to be faced with interpreting the results of a statistical procedure in the absence of information regarding the adherence of the data to the assumptions underlying the procedure. The assumptions noted by the questioner (normality and equality of variances) are only two of several fundamental assumptions underlying the valid use of multiple regression analysis as a tool of statistical inference. It is likely in the Connecticut study that these other assumptions (e.g., negligible measurement error associated with independent variables and assumption of linearity) were also not completely satisfied. Fortunately, many parametric statistical procedures, such as multiple regression, have been shown by Monte Carlo studies and other techniques to be fairly robust (or perform at an adequate level) with respect to moderate deviations from the underlying assumptions. I suspect that the deviations from the underlying assumptions associated with the Connecticut study were not severe enough have drastically altered the overall conclusions that were reported. The uncertainties regarding this issue could be obviated, however, if information regarding these important assumptions were provided in conjunction with study results.

One interesting issue that was raised concerns the fact that in the regression analysis of the San Francisco studies (*4,5*), if O/E was zero, the authors added a constant before taking logs. The dependent regression variable was then $\log[(O/E) + K]$. For the rarer tumors, many tracts would have $O/E = 0$ and all of these tracts would have $\log[(O/E) + K] = \log K$. Could this drastically affect the regression results?

It should first be pointed out that the transformation, $\log[(O/E) + K]$, was used by Kanarek et al. (*4*) for all values of O/E and not just when O/E was zero. The inappropriateness of the $\log[(O/E) + K]$ transformation was addressed by Tarter in 1981 (*6*). Tarter notes that this transformation can be written as: $\log[(O/E) + K] = \log[(O + EK)/E] = \log(O + EK) - \log(E)$. Tarter then reasons that since the argument of $\log(E)$ is

*Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261.

supposed to predict O itself and not $(O + EK)$ it is better to use the variate $\log [(0 + K)/(E + K)]$ in place of $\log [(O/E) + K]$. Tarter also goes on to show that by choosing a large value for K (specifically, $K = 32$) in his form of the transformation (rather than $K = 0.01$ as used by Kanarek et al.) that the overall significance level of the asbestos in drinking water cancer association is greatly enhanced.

One question concerned my summary table of weaknesses and asked whether it was proper in a table such as this that "uncontrolled confounding" can contribute up to eight "points," while "ecologic study design" and other items can contribute only one "point"?

I constructed Table 4 with the express purpose of summarizing the various limitations/weakness inherent in the 13 reviewed studies. The aggregation of "points" (which represents the total number of limitations/weaknesses noted) shown at the bottom of the table for each study was done for purely descriptive purposes and in no manner whatsoever influenced the overall integration of the research findings. In more statistical terms the total "points" value was considered as a nominally scaled variable rather than as an ordinal or interval scaled measure. Because of this the total "points" was not used, for example, to rank the studies from 1 to 13 on the basis of their total limitations/weaknesses. If an objective comparative analysis such as ranking were attempted it would be improper, as the questioner noted, to aggregate "points" without weighting the individual points according to their relative importance or impact.

An important question concerned whether it was really valid to assume that $p = 0.05$ or 0.10 in my large deviation probability P_D calculations? As I had pointed out in my discussion of the site summary tables, my judgment of association was subjective. To convert that type of judgment into exact p values and then to come up with P_D values such as those in Table 5 was, the questioner felt, to give the informal subjective procedure more scientific weight than it had.

It is important first to distinguish clearly the purpose and reasoning behind the large deviation probability (P_D) calculations and the calculations made to assess the level of interstudy male-female agreement (ϕ coefficients with associated Fisher-Irwin test). The P_D calculations were performed only to more objectively evaluate the extent to which the number of male and female findings to date may be due to chance rather than biologic or other factors. Such an objective assessment is always important in problems involving simultaneous inference since a certain number of statistically significant comparisons can always be expected to occur due to chance factors alone. As footnoted in the text of my paper, at the 5% level of significance the probability of falsely claiming statistical significance in at least one of n independent comparisons is $1 - 0.95^n$. Thus, the P_D values related only to the extent that the absolute number of positive findings for males and females deviate from the expected number and do not represent the level of agreement between male and female findings *per se*. Moreover, the p values of 0.05 or 0.10 chosen for the P_D calculations are not objective characterizations of subjective judgments but rather represent only the theoretical probability of observing, in any individual study, a positive finding for males or females due to chance alone, that is, under the null hypothesis that no real biologic ingested asbestos-cancer association was present. The 5% and 10% levels are consistent with the conventional Type I statistical error rates under which most epidemiologic research is performed. The resulting P_D calculations then represent the overall probability of jointly observing in $n_{..}$ independent studies, due to chance factors alone, $n_{1.}$ or more and $n_{.1}$ or more positive associations in males and females, respectively. A $P_D < 0.05$, for example, implies that there is less than a 5% chance that the absolute number of observed positive male and female findings was due solely to chance factors given a 5% (or 10%) chance of a false positive finding in any one individual study. In other words, a small P_D value argues against the likelihood that a given number of positive male and positive female findings represents only chance phenomena. However, as mentioned in my text, the P_D approach did suffer from the limitations of small sample size ($n_{..}$) and the necessity to assume that the $n_{..}$ independent studies provided qualitatively and quantitatively equivalent information toward the integration of findings for any given cancer site. For these reasons it was recommended and adopted that the results of the P_D analysis should not be regarded as conclusive assessments of risk but rather should serve only as a rough guide for the direction and emphasis of future research. In this context, the p values which were of concern earlier become essentially arbitrary since it was primarily the relative relationship of the cancer site-specific P_D values rather than their absolute magnitude which ultimately generated the recommended priority of specific etiologic hypotheses to be explored in future research. The arbitrariness of the undelying p value is evident since this prioritization or

ranking based on P_D values is completely invariant to the choice of p.

Since the P_D values do not explicitly account for the overall degree of interstudy male-female agreement, ϕ coefficients and associated Fisher-Irwin probability levels were computed and interpreted in conjunction with the P_D values. Assuming that the biologic effects of ingested asbestos are similar in males and females (i.e., a no interaction model), a good level of agreement in male-female findings (a ϕ coefficient close to unity) also argues against the likelihood that a given pattern of observed findings represents only chance phenomena. In this sense, the P_D values and ϕ coefficients are complementary; that is, for a given cancer site a low P_D value in conjunction with a ϕ coefficient close to unity is more suggestive of a true ingested asbestos–cancer association than is a low P_D value and a ϕ coefficient close to zero. Unfortunately, because of very small sample sizes, ϕ coefficients for most of the cancer sites shown in Table 5 could not be computed.

Finally, I have been asked to comment on the utility of future ecologic studies on the topic of ingested asbestos, with or without multiple regression. As noted in the text of my paper, I agree that while the ability to make a causal inference from ecologic data often can be enhanced using more sophisticated analytic techniques, such as multiple regression, there will always remain an element of uncertainty until the etiologic hypotheses generated from ecologic studies are tested more definitively at the individual level rather than at the group level.

The views and policies presented by the author in this commentary do not necessarily reflect those of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Marsh, G. M. A critical review of epidemiologic studies related to ingested asbestos. Environ. Health Perspect. 53: 49–56 (1983).
2. Harrington, J. M., Craun, G., Meigs, J. W., Landrigan, P. J., Flannery, J. T., and Woodhull, R. S. An investigation of the use of asbestos cement pipe for public water supply and the incidence of gastrointestinal cancer in Connecticut, 1935-1973. Am. J. Epidemiol. 107: 96–103 (1978).
3. Meigs, J. W., Walter, S., Heston, J., Millette, J. R., Craum, G. F., Woodhull, R. S., and Flannery, J. T. Asbestos-cement pipe and cancer in Connecticut, 1955-1974. J. Environ. Res. 42: 187–191 (1980).
4. Kanarek, M. S., Conforti, P., Jackson, L., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer incidence in the San Francisco Bay Area. Am. J. Epidemiol. 112: 54–72 (1980).
5. Conforti, P. M., Kanarek, J., Jackson, L., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer in the San Francisco Bay area 1969-1974 incidence. J. Chronic Dis. 34: 211–224 (1981).
6. Tarter, M. E. Pattern recognition in the context of an asbestos cancer threshold study. Proceedings of the 13th Annual Symposium on the Interface: Computer Science and Statistics, Springer Verlag, in press.

Environmental Health Perspectives
Vol. 53, pp. 189–190, 1983

Commentary

Additional Notes on the Case-Control Study in Western Washington on the Cancer Risk from Asbestos in Drinking Water

by Lincoln Polissar,*† Richard K. Severson*† and Edwin S. Boatman†

It is important to note that my presentation was a summary of our work and that a paper with a fuller description of procedures and detailed tables and risk estimates will be published in another journal (*1*).

One questioner asked what factors determined "no exposure" and what proportions of the cases and controls had no exposure? For each place of residence or work, the following factors could have led to an absence of exposure to Sultan River drinking water: (1) the place was located outside the boundaries of the Sultan River distribution system; (2) the place was located inside the boundaries of the Sultan River distribution system at a time when the Sultan River was not on line as a source of water for that area; (3) a subject worked at a job that, though based in the Sultan River area, involved extensive travel away from the place of employment; and (4) the subject did not use a community water supply, as water was obtained from a well, bottle or other sources. There was no estimated exposure to asbestos in drinking water in 1% of the cases and 0.6% of the controls.

The asbestos concentration in the drinking water from the Sultan River was specifically measured. Twenty-two samples were taken from the distribution system from June 1978 to April 1979. There was quite a large seasonal variation in concentration. After correction for seasonal variation, we found that the concentrations varied only randomly across the Sultan River area. The average of all uncorrected concentrations was used as the reference exposure value for work or residence episodes in the Sultan River area. A similar procedure was applied to the 73 water samples from other water sources outside the Sultan River area. The methods used for measuring asbestos concentrations are described in our companion paper. We also determined fiber length; 86% of the fibers were less than 1 μm in length.

Concerning the sensitivity of the study, we were asked why the similarity of estimated exposures of cases and controls did not reduce the power to detect risks. The similarity of estimated exposures between cases and controls appears be a consequence of the low true relative risk involved. A null finding in any epidemiologic study would manifest itself in the form of similar exposures between cases and controls. If there are true, but small differences in exposure, a much larger study would be needed to detect them.

It is important to consider that the main difference between our study and an ecologic study is the manner in which exposures were assigned to subjects. We obtained a detailed history of the residence and work location of each subject. We also asked each subject about typical water intake and use of bottled water, wells, and other water sources. Water company officials supplied us with a complete history of the Sultan River and other local water distribution systems. The result of this is that for any given date in a subject's history we could determine the level of exposure to asbestos in drinking water. The exposures were accumulated across each subject's lifetime and were, thus, highly specific per subject. In an ecologic study, all subjects residing in a specific area would have been assigned the same exposure, regardless of residence and work history and personal consumption habits.

*Fred Hutchinson Cancer Research Center, 1124 Columbia St., Seattle, WA 98104.
†School of Public Health and Community Medicine, University of Washington, Seattle, WA 98195.

We would like to respond to questions about control selection. We randomly chose living, unmatched general population controls. The sampling frame was developed by using 1970 census maps with household counts updated from recent aerial photographs. A more complete description of the controls and comparison of the quality of the data from living and deceased subjects will be available in our companion paper (*1*). We found that the accuracy of responses to key questions was similar between living cases (53% of the cases were alive at interview), next of kin of deceased cases, and controls.

We were asked why certain positive results were dismissed because of a lack of similar results for the opposite sex. The questioner asked us to consider known sex differentials in susceptibility to cancer, exposure to asbestos, and exposure to cocarcinogens. The sex-inconsistent risk for stomach and pharyngeal cancer was only one of the factors that made us conservative in our interpretation. The 95% confidence intervals for the relative risks for male pharynx and stomach cancers also approached 1.0. In addition, since many significance tests were performed in the study, the multiple comparisons problem must also be considered when reviewing results. Based on these criteria, we feel that the results for male pharyngeal and stomach cancers are not strong enough to suggest a causal relationship between these cancers and imbibed asbestos. The best course would be to include these sites in additional studies that might be carried out.

One questioner suggested that the number of cases and controls for most of the individual sites were probably too few and that it was implied in the presentation that the power was inadequate for certain sites of importance, such as the stomach and pancreas. The questioner further noted that by dismissing the apparent significant risks found for male stomach, male pharynx, and female pancreas cancers there was the implication that there was some inherent bias or lack of ability to control for confounding factors in the study. We definitely had low power to detect risks of interest for several of the sites we studied. Obviously, we cannot say anything firm about the negative results for these sites. The statistically significant results for pharynx and stomach cancers are not inconsistent with this. Low power to obtain significant results does not mean that such results will not occur, since a chance mechanism is at work. The relative risks for female pancreas ranged from 1.4 to 1.7, depending on the analysis. The smallest significance level was 0.07. The relative risks for male pancreas were less than 1.0. The bottom line is that we do not know the reason for the elevated risks for male pharynx and stomach cancers. A case-control study cannot include every possible risk factor. For most cancer sites the risk attributable to known factors is a small fraction of the total risk. Hence, in any study, unknown and uncontrolled risk factors could give spurious results due to correlation with a specific risk factor in question. The problem is inherent in the investigator's partial knowledge of nature and not in our study design.

Finally, one questioner asked us to provide a short, simple description of the statistical procedure used. Our main procedure, logistic regression, is similar to the multiple regression procedure that is commonly used in many branches of science. In ordinary multiple regression one is predicting, for example, weight from height and other factors. In our logistic regression we are estimate the probability that a subject is a cancer case (versus population control) using asbestos-in-water exposure and other risk factors. In the regression procedure a risk factor that is associated with cancer would lead to a large estimated probability that a subject who had that factor was a case rather than a control. The logistic regression procedure is highly flexible and can, for example, be used in an analysis that is similar to the Mantel-Haenszel pooled odds ratio.

The views and policies presented by the author in this commentary do not necessarily reflect those of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCE

1. Polissar, L., Severson, R. K., and Boatman, E. S. A case control study of asbestos in drinking water and cancer risk. Am. J. Epidemiol., in press.

Environmental Health Perspectives
Vol. 53, pp. 191–192, 1983

Commentary

Additional Comments on the Study of the Effect of Population Density on the Results of the California Bay Area Study

by Paul M. Conforti*

The California Bay Area studies concerning ingested asbestos and cancer have been the subject of much discussion. A number of very good questions were brought up after my presentation (*1*) and I would like to address a number of the points.

One questioner asked whether the interconnections of the water supply systems were considered as a factor in my study. I think that the interconnection of water supply systems is an important point regarding the application of asbestos fiber counts from water samples to the levels of asbestos in drinking water of census tracts. For census tracts with more than one source of water, weighted averages of asbestos fiber counts were computed. These averages were weighted for source, treatment process, pressure zone, and/or time.

The question, does the method of calculating population density take into account areas within the census tracts that were unavailable for residential purposes, such as bodies of water and public park lands, is an interesting one. The method for calculating the areas of census tracts did not take into account the portions of census tracts that were unavailable for residential purposes. The boundaries and/or areas of these portions of the census tracts were not accessible. Ultimately the areas within census tracts that were unavailable for residential purposes as well as the areas that were available for residential purposes but were uninhabited should be identified and considered in any analysis including population density.

One questioner asked what the population density criteria were for aggregating census tracts into super tracts. How large a difference in population density between previously separated tracts was permitted? Super tracts were formed by aggregating 1960 and 1970 census tracts such that the boundaries of the super tracts were identical in the two censuses. In many instances, the 1960 and 1970 census tracts were equivalent in which case no aggregation was necessary. The most frequent type of aggregation resulted from the splitting of a 1960 census tract into two or more 1970 census tracts due to increased population over the decade. Although no population density criteria were used in determining the super tracts, population changes were reflected in the population densities of corresponding super tracts.

Another question was whether the socioeconomic and occupation factors included in earlier studies were also included in these analyses? Socioeconomic and occupation variables were included in these analyses. Mean years of schooling and median family income were used as indicators of socioeconomic status. The proportion of workers in construction, electrical, and textile industries indicated the numbers of workers who might have been exposed to asbestos in their occupations. An indicator of marital status was also included in the analysis. This variable was computed as the number of unmarried persons 14 years old and over divided by the total population 14 years old and over.

I was asked to comment on the differences between my associations between asbestos indicators and cancer and the experience reported from the much larger exposures to asbestos in industry. Although I have not considered the comparisons between our results and those of studies of exposure to asbestos in industry, it would seem that since the types of exposure are different

*Group in Biostatistics, 140 Earl Warren Hall, University of California at Berkeley, Berkeley, CA 94720.

(inhalation versus ingestion), comparisons are inappropriate. Papers by Marsh and Erdreich (in these proceedings) review and compare studies of ingested asbestos. Their papers may respond to these issues in a more comprehensive way.

I would also like to comment on the differences between my population density associations and those reported by Haenszel (*2*). One of the differences between the results reported here and the population density associations with cancer reported by Haenszel et al. (*2*) is that the present study area was a single Standard Metropolitan Statistical Area (SMSA), whereas Haenszel et al. studied the entire state of Iowa. By definition the San Francisco–Oakland SMSA is relatively dense in population. The rural counties and regions of Iowa in 1950 probably were much less population dense than the "rural" census tracts of the 1970 San Francisco–Oakland SMSA. Another difference between the studies is that the Iowa results were sex-specific and age-adjusted, whereas the San Francisco–Oakland SMSA results were sex- and race-specific, age-adjusted, and adjusted for socioeconomic status, asbestos-related industries and marital status. For these reasons, the studies and their results are not really comparable.

It was brought to my attention that from data derived from a National Cancer Institute Monograph (*3*), the age-adjusted death rates for the five Bay Area counties for six digestive system cancer sites show the highest rates to be in the county with the highest population density. This county, San Francisco County, also had consistently high asbestos counts. A good question then is how was my analysis able to separate these two factors?

The regression approach yields estimates of parameters which reflect the individual contributions of the independent variables (population density, asbestos, and covariables) to the dependent variable (standard incidence ratios of cancer). Regression separates the influences of the independent variables on the dependent variable. The coefficients measure the influence of the independent variables when the values of the other independent variables are held constant. Table 12 of my paper (*1*) shows the significance of regression coefficients for asbestos and population density for cancer sites in which significant positive associations between asbestos and cancer were observed. The regression coefficients from which these data were extracted indicate that in spite of the fact that population density and asbestos were high in San Francisco county, asbestos was found to be significantly associated with cancer.

Because San Francisco County has high cancer rates, high population density, and high fiber concentrations, a question asked by several was: what consideration was given to the possibility that San Francisco dominated the results and may have biased the entire study in some way? Of the 722 census tracts in 1970, 212 were reported as having 10^7 or more asbestos fibers per liter of drinking water. San Francisco County accounted for 149 of these high fiber concentration census tracts. The other 63 census tracts, 30% of the total high fiber concentration census tracts, came from Alameda and San Mateo Counties. The highest concentrations of asbestos in drinking water were found in San Mateo County. Chi-square tests for linearity were used to evaluate the linearity of the association between asbestos and cancer for four census tract groupings of asbestos. These tests revealed no significant deviation from linearity. A lack of linearity might have indicated an anomaly in the study area due to some unmeasured variable or variables. However, none of the tests for white males showed nonlinearity and only breast, female reproductive, and urinary cancers for white females indicated a departure from linearity for asbestos and cancer. Due to the linearity of the associations between asbestos and cancers and since a large proportion of high fiber concentration census tracts existed outside of San Francisco County, it was concluded that no area in the study dominated the results.

The views and policies presented by the author in this commentary do not necessarily reflect those of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Conforti, P. M. Effect of population density on the results of the study of water supplies in five California counties. Environ. Health Perspect. 53: 69–78 (1983).
2. Haenszel, W., Marcus, S. C., and Zimmerer, E. G. Cancer Morbidity in Urban and Rural Iowa. Public Health Monograph No. 37, U.S. GPO, Washington, DC, 1956.
3. Mason, T. J., McKay, F. W., Hoover, R., Blot, W. J., and Fraumeni, J. F., Jr. Atlas of Cancer Mortality for U.S. Counties: 1950–1969. NCI Monograph, U.S. GPO, Washington, DC, 1975.

Environmental Health Perspectives
Vol. 53, pp. 193–194, 1983

Commentary

Answers to Some Questions Raised by the Presentation on the Graphical Analysis of the Interrelationships Between Waterborne Asbestos, Digestive System Cancer and Population Density

by Michael E. Tarter*

Two interesting questions were raised following my presentation (*1*), and I am pleased to have the opportunity to clarify these issues. The first question dealt with the data in Figure 1. The questioner asked about the interpretation of the axes as they appeared on the graph. In answer to his question it is important to note that all graph coordinate axes pass through the x and y sample means of the displayed data. For example, the mean tract number of the frequency diagram shown in Figure 1 is 216, while the mean number of individuals per square mile is 5270. Thus, the two lines shown in Figure 1 are $X \equiv 216$ and $Y \equiv 5270$ (the symbol "≡" is the usual mathematical notation for the phrase "is identical to").

The program that we use, GRAFSTAT, is designed to perform certain scaling and positioning operations automatically. Hence, it applies an initial pass through the data to compute the range of the (X,Y) observations (from which the position of the graph's boundaries are calculated). Simultaneously, it calculates the pair $(\bar{X},\bar{Y})$ (from which the axes are positioned), and the standard deviation units. For example, the very small number "2" which appears slightly below the midpoint on the Y-axis of Figure 1, is 2 y standard deviation units from the mean point $(\bar{X},\bar{Y})$.

For normally distributed data, as the sample size increases one would of course expect that the number x of all measurements which lie between plus and minus 1.95 standard deviation units of the mean would approach the value x = 5%. The fact that the data depicted in Figure 1 extends more than five standard deviation above, but less than one standard deviation unit below, the point $(\bar{X},\bar{Y})$ suggests a high degree of skewness for the population density variate. On the other hand, the fairly even spread of data on either side of the mean point in the $\pm\bar{X}$ direction suggests a fairly uniform distribution of the tract number variate.

In the second question I was asked how I concluded that the data in Figure 23 appeared to be composed of two population subgroups of the analysis displayed in Figure 25. The questioner thought that they appeared to be an artifact caused by the sparse data in this region and by the smoothing function employed.

This is a question about smoothing and bifurcation. The method used to smooth the displays shown in Figure 25 is based on the modifications of the Doetsch-Fourier transform technique for spectral decomposition as modified by Tarter and Silvers (*2*). As I stated in this paper, "the choice of λ within Λ of (2.6) will not affect the variances of any component." Parenthetically, it should be mentioned that despite the fact that the 27 references listed at the back of this paper suggest considerable interest in the λ method, there has been in the last 8 years no contention to the above assertion. This assertion implies that one can separate or smooth distributional components in one direction with absolutely no effect on the components in any other direction.

*Department of Biomedical and Environmental Health Sciences, University of California at Berkeley, Berkeley, CA 94720.

It should be mentioned that the actual λ value chosen was selected for the purpose of smoothing as opposed to separating components. One would, therefore, expect that if it had any spurious effect (which it does not), the λ method would tend to hide rather than emphasize distributional components. As discussed in one of my earlier works (*3*), benchmark data confirmation procedures have been used to create a series of test patterns. When the λ smoothing procedures were applied in conjunction with these test patterns, no spurious curve bifurcation was uncovered.

The views and policies presented by the author in this commentary do not necessarily reflect those of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Tarter, M., Cooper, R. C., and Freeman, W. R. Agraphical analysis of the interrelationships among waterborne asbestos, digestive system cancer and population density. Environ. Health Perspect. 53: 79–90 (1983).
2. Tarter, M., and Silvers, A. Implementation and applications of bivariate Gaussian mixture decomposition. J. Am. Statist. Assoc. 70: 47–55 (1975).
3. Tarter, M. Pattern recognition in the context of an asbestos cancer threshold study. In Computer Science and Statistics: Proceedings of the 13th Symposium on the Interface (W. F. Eddy, Ed.), Springer-Verlag, New York 1981, pp. 105–110.

Environmental Health Perspectives
Vol. 53, p. 195, 1983

Commentary

Additional Notes on the Use of Quantitative Analysis of Urine To Assess Exposure to Asbestos Fibers in Drinking Water in the Puget Sound Region

by Edwin S. Boatman*

I am happy to answer some questions that were raised following my presentation. I was asked for the ranges in counts per grid opening of the control water and urine analyses which appeared in Table 2. For Everett residents the counts for the urine samples ranged from no fibers in 20 grid openings to 2.0 fibers per grid opening (FGO) and 0.15 to 1.5 FGO for the control waters. In Seattle-Bellevue the counts for urine samples ranged from no fibers in 20 grid openings to 1.2 FGO and 0.15 to 0.45 FGO for the control waters.

I was also asked why I did not correct the urine data in Table 2 for the control counts before comparing long versus short residence time fiber concentrations in the urine. An inherent problem with the urine analysis that is distinct from the control water analysis is that often a residue of mucus material masks some unknown number of the asbestos fibers on the surface of the grid. Secondly, less fibers from the urine tend to give positive selected area diffraction patterns than do fibers from the control waters. Thus I did not think that the control water "background" could be directly subtracted from the urine analysis values. I agree that the control water counts are a function of the amount of water placed in the control container, but it should be noted that the amount of control water filtered was chosen to be comparable with the volume of urine filtered. Certainly the optimum methods for taking into account the "background" contamination in the analysis of urine for chrysotile remain to be determined. However, I do not think that any other way of expressing the control water data would change my conclusion that within the limits of sensitivity of the present methods we found no excess of chrysotile asbestos fibers in the urines of persons ingesting over 100 million fibers/L in their drinking water.

The views and policies presented by the author in this commentary do not necessarily reflect those of the U.S. Environmental Protection Agency. Mention of trade names or commerical products does not constitute endorsement or recommendation for use.

*Department of Environmental Health, University of Washington, Seattle, WA 98195.

Environmental Health Perspectives
Vol. 53, pp. 197–200, 1983

Accumulation of Ingested Asbestos Fibers in Rat Tissues Over Time

by Kusum Patel-Mandlik*† and James Millette‡

With the use of the transmission electron microscope, asbestos fibers have been assessed in kidney cortex of four groups of rats previously exposed to intermediate-range feeding grade chrysotile asbestos. Newborn rats, from mothers gavaged with asbestos during pregnancy, were gavaged twice a week at the dose level of 50 mg/kg beginning at age day 7 until their natural death or sacrifice. The rats were divided into four groups by age: 0-200, 200-400, 400-600 and 600-800 days. Of the 20 rats comprising the four test groups, 17 were positive, average fiber recovery being 5.34 × 10^3/mg dry weight. Average fiber level in control tissues was 0.23 × 10^3/mg dry weight. Fiber recovery in tissues from control animals was shown to be significantly lower ($p < 0.005$) than that from test tissues. Test groups showed highly significant differences ($p < 0.005$) from each other in the fiber levels. Dose-response relationship was not significant ($0.05 < p < 0.1$). The length distribution and the alterations in morphology of the recovered fibers are described. This study is consistent with the passage of chrysotile asbestos across the gastrointestinal wall.

Introduction

A relationship between ingested asbestos and cancer incidence in humans was suggested (*1–3*) through epidemiological studies. Recovery of fibers in tissues and urine of humans exposed to asbestos has been reported (*4–7*). Short-term animal feeding studies (*8–12*) have shown that ingested fibers penetrate, migrate between, and are recoverable from gastrointestinal tract, lymph, distant tissues and urine.

The present study was undertaken to investigate whether penetration, migration and retention occur in tissues of animals chronically exposed to asbestos. Rats employed in a carcinogenicity study (National Cancer Institute Grant 21684, unpublished) were used. Kidney cortex was selected, since in our past study with baboons, the highest concentration of fibers was found in kidney cortex among ten organs under analysis (*12*). Selikoff (*5*) reported excessive kidney cancer among insulation workers, although numerous asbestos fibers were recovered in liver. The relationship of fiber concentration in tissue with the time of exposure was tested in the present study by using rats gavaged for various time durations.

Materials and Methods

Sprague-Dawley rats were gavaged with Environmental Health Sciences Sample No. 109C feeding grade intermediate-range chrysotile asbestos. The dosage and gavage schedule were as described earlier (*13*). Rats dying of natural causes between ages 0-800 days were grouped into four groups by age ranges at death: namely, 0-200 days, 200-400 days, 400-600 days and 600-800 days. Kidney tissues were collected from 20 randomly selected rats, five per age group. Four control rats, one in each age group, were randomly chosen to represent the level of asbestos in control tissues.

Tissue preparation for electron microscopic analysis was as described earlier (*12*). A Phillips 300 transmission electron microscope was used for chrysotile identification and for counting and sizing the fibers. The number of chrysotile fibers per grid hole followed a Poisson distribution (*14*); 50 grid holes were examined per sample, thus

*Environmental Health Sciences, School of Public Health, University of Illinois at Chicago, P.O. Box 6998, Chicago, IL 60680.

†Present address: Indian Institute of Toxicology, 172/74 Hornby Building, D. N. Road, Fort, Bombay, India 400 001.

‡Health Effects Research Laboratory, Environmental Protection Agency, 26 W. St. Clair Street, Cincinnati, OH 45268.

reducing the probability of a zero count to less than 5%. The detection limit of our analytical technique (*13*) was 88 fibers/mg dry weight if one fiber was found in 50 grid holes and if $L = 1$, where L was the weight of tissue (mg) per grid surface. Using the same preparative and counting techniques, blank preparations and tissue preservative were tested for the background level of fibers.

Characteristic morphology of chrysotile was revealed by some but not all fibers. Hence, fibers recovered in our tissues were classified as Class A, B, C, and D fibers according to definitions based on personal judgment of the morphological appearance of fibers (*13*). Blind-coded analysis was performed on samples from each test category on representative grids at the Health Effects Research Laboratory of the U.S. Environmental Protection Agency, Cincinnati, OH.

Among additional organs, lung and omentum of a test rat were analyzed.

Results

When the method described was used, no problem was encountered in grid preparation. Transmission electron microscopic examination was confidently reliable due to minimal residue or debris on grids. The average fiber recovery of Class A + B + C fibers in test samples was 788 or 5.34×10^3/mg dry weight, whereas that in control samples was 10 or 0.2×10^3/mg dry weight. Fibers were at below detection level in blank grids (prepared to test preparative contamination) and grids prepared from tissue preservative.

The highest concentration was found in samples from rats in age range 400-600 days. Tissues from rats in age range 0-200 days showed the lowest concentration (Table 1). For statistical comparisons, square-root transformations of data were used because the mean and the variance were proportional. Fiber recovery in test and control groups were statistically (*15*) for each of the four time intervals, and each showed significant difference at $p < 0.005$. The individual transformed data were combined to test an overall difference between test and control which was found to be significant at $p < 0.005$.

The dose-response effect was tested by comparing the fiber recovery in test samples from the four age groups. Group I animals (0-200 days) represented the lowest dosage of gavaged asbestos and group IV animals (600-800 days) represented the highest dosage, since, the older the animal, the higher the cumulative dosage it would have. The statistical evaluation of transformed data by weighted regression analysis (*16*) revealed that when Class A + B fiber counts were considered, no linear relationship was found at the 5% level of significance. However, when Class A + B + C fiber counts were considered, although there was no linear relationship between the age of animal and number of fibers, a quadrate relationship was found between the variables at $p < 0.08$.

Bundles and clusters of fibrils were recovered in all age ranges of rats to form 25.8% of total fibers. Fiber dimensions were measured; however, only length data were reported (*13*). Diameter was not meaningful, due to swelling or lack of sharp edges in some degraded fibers. The length varied from 0.08 to 5.71 μm, where the median ranged from 0.23 to 0.46 μm among the fibers in four groups of rats. The frequency of length distribution (Table 2) of Class A and B fibers combined was unimodal for rat groups I and III, bimodal for rat group IV and trimodal for rat group II. The mean values of four groups showed bimodal distribution. Fiber lengths and frequency distribution of fiber Classes A, B, C, and D recovered in representative samples from test rats in age groups II, III and IV have been described in tabular and nomogram forms (*13*).

One bundle of fibrils was recovered in omentum, and none in lung of one test rat.

Table 1. Fiber counts in the kidney cortex from rats gavaged with chrysotile asbestos.

Age group of rats[a]	Average kidney weight, mg	Chrysotile fibers/mg dry weight × 10^3 in each class of fibers[b,c]		
		A + B	C	A + B + C
I	1230	1.24 ± 1.1 (BDL)	0.33 ± 0.3 (BDL)	1.57 ± 1.4 (BDL)
II	1722	2.66 ± 1.7 (BDL)	2.71 ± 2.0 (0.53)	5.37 ± 4.0 (0.56)
III	1870	8.00 ± 4.2 (BDL)	1.62 ± 0.6 (BDL)	9.63 ± 4.1 (BDL)
IV	2040	3.31 ± 1.2 (BDL)	1.47 ± 0.9 (0.34)	4.78 ± 2.0 (0.34)

[a]Each group consisted of five rats.
[b]Fiber classes are defined in the text.
[c]Fiber counts in kidney cortex of control rats are shown in parentheses; BDL denotes below detection level.

Table 2. Frequency of length distribution of fibers recovered in kidney cortex of gavaged rats.

Length range, µm	Fiber distribution, %[a]					
	Group I rats	Group II rats	Group III rats	Group IV rats	Mean	Cumulative
0–0.5	84.6	56.3	79.0	89.8	77.4	77.4
0.5–0.99	7.7	22.3	15.8	7.8	13.4	90.8
1.0–1.7	3.9	3.0	3.8	0.6	2.8	93.6
1.8–3.1	3.8	8.7	1.4	0	3.5	97.1
3.2–5.5	0	9.7	0	1.2	2.7	99.8
5.6–9.9	0	0	0	0.6	0.15	100.0

[a]Chrysotile fibers A + B sized by transmission electron miscroscopy.

Discussion

Penetration of ingested asbestos fibers through gastrointestinal tract and recovery of migrated fibers in distant tissues have been reported in animal and human studies (*6–13, 17–19*). Contrary to this, other studies (*20–22*) indicate that ingested fibers do not penetrate or migrate. In the present study, recovery of chrysotile fibrils, bundles, and clusters in kidney cortex of gavaged rats clearly shows that migration occurs, and it supports our previous findings of chrysotile asbestos recovery in kidney cortex of a baboon (*12*) and humans (*23*). Since the clusters contained one or more bundles, 57 bundles were encountered within 39 clusters. Bundles formed 29.2%, and bundles and clusters formed 25.8%, of total recovery. Recovery of fibers in 17 out of 20 rats suggest that some animals may resist fiber penetration and/or migration. Biological variation was suggested by Sebastien et al. (*17*) in their studies with rats.

The presence of fibers in test samples was obviously not artifactual, since the results were based on up to 250 observations per age group of rats, and the background contamination from air, reagents and preparative procedures was minimal or below detection level. One gavage study (*22*) failed to demonstrate penetration and migration of ingested fibers due to contamination, resulting in fiber counts in the controls in the same range as the counts in test baboon blood and urine. Moreover, the detection limit of their methodology was low due to scanning of only 20 grid holes per sample and use of only one test animal.

By using square root transformed data on fibers of Class A + B or Class A + B + C a significant difference at $p < 0.005$ was found between the fiber concentrations in test and control tissues. Controls were compared with each test group separately, as well as with the four test groups combined. Fiber concentrations within the four age groups showed a statistically significant difference ($p < 0.005$).

A further aim of this study was to determine whether there was a relationship between the amount of ingested chrysotile asbestos and concentration of fibers retained. There was a rise in fiber level in the tissues from group I to group III rats, followed by a drop in the tissues from group IV rats (Table 1). Rat group IV received cumulatively more fibers than rat groups III, II or I because the rats in this study were gavaged twice weekly at the dosage of 50 mg asbestos/kg body weight until death. No linear relationship w found. On accepting statistical significance fo values < 0.1, the quadratic model of regression analysis revealed a relation ($p < 0.08$) between dosage and number of fibers of Class A + B + C retained in the kidney cortex. The quadratic relationship could signify time-related biological causes of low fiber recovery, such as degradation of fibers beyond TEM identity and elimination of fibers in feces and urine (*6,18,19*). No relationship was observed with Class A + B fiber counts.

The length of chrysotile asbestos Class A + B fibers recovered in test tissues showed a skewed distribution. Shorter fibers outnumbered the longer fibers. All the fibers were less than 9 µm in length (Table 2). Most of the recovered fibers were in the length range of 0.31–0.5 µm. It is not known whether shorter fibers selectively penetrate and disseminate or the fibers break into shorter lengths during the journey from oral c try into kidney cortex through the blood system. The health significance of such a large number of retained short-length fibers is not known. Hamilton et al. (*24*) reported that a sample containing chrysotile fibers shorter than 0.5 µm was ineffective in coagulation of plasma. There was no particular pattern of fiber length in relation to the age group or rats (Table 2), except that fewer fibers recovered in group II tissues were in the length range of 0-0.5 µm than in the other groups and showed trimodal distribution.

Morphological degradation was used as a criterion to form Classes A, B, C, and D of the recovered fibers. It was not possible within the scope of this study to use microprobe analysis for more precision. Degraded fibers have been reported previously (*12,19*) in gavage studies. It is suggested that there might be a degradative mechanism associated with the residence time of fibers *in vivo*. It is possible that during their passage and stay within the body system, fibers could be influenced by the body fluids, temperature, agitation and other factors.

Assuming that the degradative mechanisms affecting the morphology and diffraction property of the chrysotile preclude positive identification of all of the fibers present on the grid surface, the results expressed in fibers/milligram dry weight could be considered to be conservative measures of tissue fiber concentration. Mass concentration was not computed, since it is often very inaccurate because of the poor counting statistics associated with large fibers (bundles, clusters) that are fewer in number but represent most of the actual mass. Recovery of bundles and clusters suggests that, besides single fibrils, other forms could penetrate and migrate; that fibers accumulate in the same area of the tissue; or that the preparation procedure causes some fibrils to clump together.

Results from analysis of a test rat's lung and omentum were considered insufficient to derive information about fiber retention in these organs.

The authors are grateful to Drs. S. S. Epstein and Z. M. Iqbal of the University of Illinois at Chicago for providing the rat tissues, and to Drs. Claparols and T. Raju for the gavage, autopsy and related functions. Thanks are due to Dr. M. K. Patel for expert assistance in statistical analysis.

This work was supported by U.S. Environmental Protection Agency Cooperative agreement No. CR 807289-01-0

The views and policies presented by the author in this commentary do not necessarily reflect those of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Keal, E. S. Asbestosis and abdominal neoplasms. Lancet ii: 1211–1216 (1960).
2. Selikoff, I. J., Churg, J., and Hammond, E. C. Asbestos exposure and neoplasia. J. Am. Med. Assoc. 188: 22–26 (1964).
3. Selikoff, I. J. Epidemiology of gastrointestinal cancer. Environ. Health Perspect. 9: 299–305 (1974).
4. Langer, A. M., Selikoff, I. J., and Sastre, A. Chrysotile asbestos in the lungs of persons in New York City. Arch. Environ. Health 22: 348–361 (1971).
5. Selikoff, I. J., Hammond, E. C., and Seidman, H. Mortality experience of insulation workers in the United States and Canada, 1943–1976. In: Health Hazards of Asbestos Exposure (I. J. Selikoff, and E. C. Hammond, Eds.), Ann. N.Y. Acad. Sci. 330: 91–116 (1979).
6. Cook, P. M., and Olson, G. F. Ingested mineral fibers: elimination in human urine. Science 204: 195–198 (1979).
7. Carter, R. E., and Taylor, W. E. Identification of a particular amphibole asbestos fiber in tissues of persons exposed to a high oral intake of the mineral. Environ. Res. 21: 85–93 (1980).
8. Cunningham, H. M. Moodie, C., Lawrence, G., and Pontefract, R. D. Chronic effects of ingested asbestos in rats. Arch. Environ. Contam. Toxicol. 6: 507–513 (1977).
9. Patel-Mandlik, K. J., Hallenbeck, W. H., and Millette, J. R. Asbestos fibers. 1. A modified preparation of tissue samples for analysis by electron microscopy; 2. Presence of fibers in the tissues of a baboon fed chrysotile asbestos. J. Environ. Pathol. Toxicol. 6: 1385–1395 (1979).
10. Patel-Mandlik, K. J. Distribution of orally administered chrysotile asbestos in newborn baboon body. U.S. Environmental Protection Agency, 1980, EPA 600/1-80-022.
11. Sebastien, P., Masse, R., and Bignon, J. Recovery of ingested asbestos fibers from the gastrointestinal lymph in rats. Environ. Res. 22: 201–216 (1980).
12. Patel-Mandlik, K. J., and Millette, J. R. Evidence of migration of ingested asbestos into various baboon organs. Scanning Electron Microsc. 1: 347–355 (1980).
13. Patel-Mandlik, K. J., and Millette, J. R. Chrysotile asbestos in kidney cortex of chronically gavaged rats. Arch. Environ. Contam. Toxicol. 12: 247–255 (1983).
14. Cochran, W. G. Some methods for strengthening the common chi-square tests. Biometrics 10: 417–451 (1954).
15. Sokal, R. R., and Rohlf, F. J. Introduction to Biostatistics. W. H. Freeman. San Francisco, 1973, pp. 286-304.
16. Dixon, W. J. Biomedical Computer Programs—BMDP 1R. University of California Press, Berkeley, 1975, pp. 453–489.
17. Westlake, G. E., Spjut, H. J., and Smith, M. N. Penetration of colonic mucosa by asbestos particles: An electron microscopic study in rats fed asbestos dust. Lab. Invest. 14: 2029–2033 (1965).
18. Cunningham, H. M., Pontefract, R. D., and O'Brien, R. C. Quantitative relationship of fecal asbestos to asbestos exposure. J. Toxicol. Environ. Health 1: 377–379 (1976).
19. Hallenbeck, W. H., and Patel-Mandlik, K. J. Presence of fibers in the urine of a baboon gavaged with chrysotile asbestos. Environ. Res. 20: 335–340 (1979).
20. Gross, P., Harley, R. A., Swineburne, L. M., Davis, J. M. G., and Green, W. B. Ingested mineral fibers: Do they penetrate tissue or cause cancer? Arch. Environ. Health 29: 341–347 (1974).
21. Bolton, R. E., and Davis, J. M. G. The short-term effects of chronic asbestos ingestion in rats. Ann. Occup. Hyg. 19: 121–128 (1976).
22. Hallenbeck, W. H., Markey, D. R., and Dolan, D. G. Analyses of tissue, blood, and urine samples from a baboon gavaged with chrysotile and crocidolite asbestos. Environ. Res. 25: 349–360 (1981).
23. Patel-Mandlik, K. J. Asbestos fibers in normal and cancerous human kidneys. Arch. Environ. Contam. Toxicol. 10: 47–54 (1981).
24. Hamilton, J. A., Chan, J. Y., and Movat, H. Z. Coagulation of human plasma by asbestos fibers. Environ. Res. 26: 119–124 (1976).

Environmental Health Perspectives
Vol. 53, pp. 201–204, 1983

Summary of Discussion Sessions: Workshop on Ingested Asbestos

by James R. Millette*

At the Workshop on Ingested Asbestos, October 1982, the technical papers were divided into five topic areas: toxicology, epidemiology, gastrointestial tract penetration, *in vitro* testing and engineering methods. Following the presentations in each topic area, a question and answer discussion period was held. A number of important issues were raised and statements clarified during this part of the program. Unfortunately all questions, answers, and comments could not be included in these Proceedings. This paper will attempt to summarize the points of general interest. I would personally like to thank all those at the Workshop who participated in making the discussion sessions constructive, worthwhile exchanges of ideas.

Following the session on toxicology, a question was asked regarding the appropriateness of the animal models used in the NIEHS (National Institute of Environmental Health Sciences) feeding studies. Specifically the question asked was: are there inhalation or injection studies that show a positive carcinogenic response from asbestos in both the F344 rat and Syrian golden hamster? Dr. L. Condie (U.S. EPA) responded that the intratracheal placement of asbestos fibers had been known to produce tumors at a high percentage as early as 16 months. Dr. E. McConnell (NIEHS) also responded by stating that tumors attributed to asbestos fibers had been produced in both animal models by routes of administration other than ingestion. He cited the work of Smith with hamsters and recent work on the effects of inhalation and intrapleural inoculation of asbestos in rats presented by Wagner at the Meeting on the Biological Effects of Man-made Mineral Fibres in Copenhagen, April 20-22, 1982.

J. Lee (Cincinnati) asked whether it was possible that the normal lifespan of the rodents too short to produce significant results. Dr. McConnell answered by saying that it is well accepted by most people who study geriatric disease, that the aging phenomena in the rat parallel those in other mammals. While the rat lives for only two years, it goes through the same steps of aging that people go through in 70 years. Dr. D. Coffin, U.S. EPA, commented that it seemed to him that there was no reason to believe that tumors which may have been derived from the gastrointestinal route of entry in man had any greater latency period than those which had been derived from the respiratory tract, the lung or the pluera. He felt that the rat was sufficiently long-lived to develop these tumors in the pluera and the lung and that by analogy, that the rats probably live long enough to develop a tumor of such organs as kidney, colon, etc., as might expected from the GI tract port of entry. Both McConnell and Dr. Condie commented further that to reproduce the study with primates would take about 30 years and require at least $40 million. The NIEHS rodent study cost about $4 million.

There was some discussion raised by E. Meek (Department of Health and Welfare, Canada), Dr. W. Meigs (Yale University) and Dr. M. Tarter (University of California—Berkeley) on the effect of the type of diet used in the animal experiments. The animals' diet in the NIEHS study was a standard animal chow which is low in fat, high in natural fiber and optimum in nutrients, whereas humans tend to have a high fat diet. There was also a question raised by P. Tsai (Region I, U.S. EPA) about the effect of asbestos exposure on animals with damaged intestinal tracts, as in the case of an ulcer. Dr. McConnell responded by acknowledging that whether to use a healthy animal with an optimum diet or a diet consisting of junk food similar to the diet of many humans was a question that had been argued for years. It

*Toxicology and Microbiology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, 26 West St. Clair, Cincinnati, OH 45268.

was his opinion that one must use the cleanest animal and most optimum diet possible to provide controlled tests that can be compared properly. He felt that when one is trying to compare the carcinogenicity of chemical X versus chemical Y, fairly standard protocols are necessary. Use of a diet deficient in some nutrient, while perhaps a valid experiment, would produce results difficult to interpret and impossible to use in comparisons with other studies. Dr. Condie pointed out that several of the studies in the literature used high fat diets (such as asbestos in butter) and saw no difference in results from the NIEHS studies.

Mrs. H. Penetrack (Blairsville, PA) asked if the fibers used in the NIEHS feeding study were smaller than those that can be released into the water by deteriorated asbestos-cement pipe. On the basis of our EPA studies, I was able to respond that the fibers used in the NIEHS feeding studies were, in general, much longer than the fibers that we found in the drinking water systems. If anything, there was a higher percentage of longer fibers in the feeding studies' materials than would be found in any drinking water samples.

S. Hayward (California State Health Department) asked about the physical characterization of the fibers used in the NIEHS feeding study. Dr. McConnell responded that a characterization of the fibers can be found in a 1980 Bureau of Mines Report (#8452). Dr. P. Cook (U.S. EPA) cautioned that it is difficult to know much about the dose while in the body. While 1% in the diet is a very large dose, if the majority of fibers are held in very tight, large clumps, the exposure to individual fibers may be much lower. Dr. McConnell remarked that Sebastien had used the same chrysotile in his studies and showed that the material was absorbed or penetrated into the lympatics. It is interesting to note that Sebastian and his co-workers also looked at the fiber sizes of the chrysotile materials after dissolving the feed pellets. They found that the short-range chrysotile had an average length of 1.8 μm and that 10% of the fibers were over 4 μm in length. This compares well with the data in the Bureau of Mines Report that shows that the average fiber length was 2.1 μm and that the same percentage (10%) of the fibers was over 4 μm in length.

L. McCabe (U.S. EPA) asked Dr. McConnell whether he felt the toxicology study had been done as well as it might have been. Dr. McConnell responded that he felt that the rat study had been one of the cleanest studies with which he had ever been associated. He cited the main flaws in the study as not recording food consumption or weight gain data between 3 and 8 weeks of age and having too high a rate of intestinal neoplasia with the suspect cocarcinogen. With the hamster study he felt that a more aggressive sacrifice policy would have allowed interpretation of some subtle lesions lost because of early autolysis. One flaw of the hamster study was that not enough dimethylhydrazine was used in order to test cocarcinogenesis.

Following the session on epidemiology, Dr. Coffin commented that, with a mean latency period of 30 yr. for asbestos disease of the G.I. tract, it would be a minimum of 20 yr. before an appreciable effect would be seen. Dr. R. Cooper (University of California—Berkeley) commented that they were able to establish with some reliability that the water quality had been basically the same in most districts of the Bay Area for up to 40 yr. Dr. L. Erdreich (U.S. EPA) showed a table indicating that Duluth had less than 20 yr. exposure and that the second study in Connecticut had considered a group exposed for 20-25 yr. Dr. L. Polissar (Fred Hutchinson Cancer Research Center) commented that there was a need to pay attention not only to how long certain areas had been getting water but also to how long people had been staying in that particular area. He remarked that there are probably places in Europe where people have been getting their water from the same source for 400 yr., but there would be very few people with 400 yr. of exposure. He felt that most areas of the United States are highly migratory. When he looked at the number of people whose actual exposure to the Sultan River water was over 40 yr., only 25% of the cases and controls had that amount of exposure. He considered that that percentage was limiting, even in a case-control study the size he had with almost 400 cases. He thought that the case-control approach could work in other areas if you could get hundreds, or possibly thousands, of cases. He was not sure what the requirements for a cohort study would be but thought that they might be even more prohibitive.

Dr. Meigs commented that in connection with the types of cancers which might result from asbestos ingested through water supplies, one should consider peritoneal mesothelioma as a possible indicator. He had studied all reported cases of mesotheliomas in Connecticut over the past 45 yr., with special emphasis on the period from 1955 to 1977, and found that the predominant form of mesothelioma was pleural. Only two men over 70 had peritoneal mesothelioma. However, when he looked at the distribution of mesotheliomas in men between the ages of 30 and 49, it turned out that, although there were not very many cases, 7

of the 20 cases were peritoneal; there were thus more peritoneal cases in the younger people than in the older ones. Only a few of the cases had evidence of occupational asbestos exposure. When compared with data on fiber counts in water supplies, however, the peritoneal cases, in both men and women, showed no association with asbestos in the water supplies.

Dr. J. Cotruvo (U.S. EPA) asked about the variability of asbestos fiber concentrations in the various water supplies over time. Dr. Polissar indicated that the turbidity of the Puget Sound area supply was higher in the past and that fiber counts appeared to be proportional to turbidity. Dr. M. Kanarek (University of Wisconsin) replied that in the California study some water barrels had been found in fall-out shelters which were marked by water source and date collected, so that some actual water samples were ≥ 20 yr old. He also indicated that the variability in sampling results caused by a drought during the sampling period could not be completely assessed. S. Hayward commented that he had seen a very large seasonal variation dependent on rainfall and would expect that the drought did affect the fiber concentrations. Dr. Cooper agreed that the fiber counts in the Bay Area water supplies were seasonally affected and suggested that during the drought, the counts were lower than typical. He commented that during the wet season, heavy rains resulting in increased run-off into reservoirs and winter winds which turned reservoirs over caused high fiber counts to occur. He had seen this in Marin County during nondrought years, at which time the fiber counts were very high. He felt that his group had not been able to do repeated fiber counts over extented periods of time because of high costs per sample and the human limitations of their microscopist. Dr. Cotruvo commented that he thought that the problem was not so much the absolute values, but the relative values among census tracts which were important. If during the drought, the reservoirs were putting out lower than typical concentrations, but the contribution from other sources like asbestos pipe was either lower or higher, then the correlations could vary. Therefore, he felt it was not so much the absolutes but the variability of relative amounts which could affect the studies.

Following the session on the penetration of asbestos fibers through the gastrointestinal tract, several researchers, E. Meek, P. Tsai and Dr. W. Hallenbeck (University of Illinois) and Dr. E. Boatman (University of Washington) commented on the importance of having low contamination levels in the studies. Dr. Cook commented that it is easier to use amphibole fibers for penetration studies rather than chrysotile because chrysotile is more of an ambient contaminant. Dr. Cook also commented that his initial work with human urine indicating that a fraction of approximately one fiber in 1000 penetrated did not seem reasonable, and after having done more work on urine, he concluded that the number would be approximately one fiber in 10,000. The French workers, Sebastien and Bignon, have reported numbers such as one fiber in 100,000 penetrating. Dr. Cook also stated that he wanted to re-emphasize the importance of the properties of the individual types of particles on their possible biological behavior. He concluded that chrysotile was far less durable than the amphibole fibers and that synthetic fibers, such as fibrous glass, had a 100-fold lower durability than asbestos fibers.

Based on the points brought up during the presentation of Dr. K. Seshan (University of Arizona), Dr. Polissar wondered if changes in fiber properties after exposure to water and stomach acid could explain the fact that inhaled asbestos was carcinogenic and the imbibed asbestos did not appear to be in some of the studies. E. Meek commented that the University of Sherbrooke had been doing some *in vitro* work on the effects of acid treatment of fibers and their hemolytic potential. They had found that the hemolytic potency seemed to decrease with acid exposure but she did not think that anyone would be prepared to say how that correlates with carcinogenicity.

Following the session on *in vitro* testing, Dr. McConnell asked for an explanation of what could be initiating mesothelial cells if asbestos is only a promoter or cocarcinogen. Dr. B. Mossman (University of Vermont) replied that she thought that asbestos was a promoter in mesothelioma but also could serve as an initiator, because there was no evidence suggesting, for example, a synergistic effect of smoking on mesothelioma. Therefore, hydrocarbons do not appear to be initiators in that case. She emphasized that the agents that Stanton found to be most carcinogenic were fibrous, not particles. Fibers were also agents (which she found selectively, in comparison to particulates) that cause cell division. She thought that an initiating effect could be seen which was a foreign body or a physical type of carcinogenesis in mesothelioma, whereas the initiator was a chemical carcinogen in bronchogenic carcinoma. Dr. B. Daniel (U.S. EPA) added that if a fiber was capable of not only initiating a break in the chromosome, but causing hyperplasia as well, it

could be acting both ways. He commented further that the evidence for asbestos acting as a classical genotoxicant was weak, however.

J. Jackson (Asbestos-Cement Pipe Producers Association) asked if Dr. Mossman had reviewed the work done by Dr. Flowers in coating chrysotile asbestos and ameliorating the surface charge. Dr. Mossman replied that she had reviewed a description of the process that has been suggested as a way of modifying the biological effects of asbestos and making it less cytotoxic. She thought that it was an attempt in the right direction but did not believe there was any evidence suggesting that because the treated asbestos fibers are less cytotoxic that they would be less carcinogenic.

Dr. Daniel commented that a recent publication by Dr. Mossman showed that the fibers do not affect the metabolism of benzo(a)pyrene. In other words, the damage to the DNA adducts, resulting from the treatment of hamster tracheal cultures with benzo(a)pyrene and asbestos were identical to those obtained in the absence of the fiber. Dr. Daniel had obtained similar results in a collaborative study with Dr. R. Stevens and L. Joseph of Ohio State University, using cultured human fibroblasts. The BP-DNA adducts were essentially the same. No differences were observed in the actual metabolites or in the extent of metabolism of BP by these human fibroblasts in the presence or absence of the NIEHS short or intermediate chrysotile. Further, he had also studied metabolism by 7,12-dimethybenz(a)anthracene, which has exocyclic methyl groups and is thus subject to yet another form of metabolism, the formation of hydroxymethyl groups. With all five UICC fibers using a rat liver microsome system, he found no difference in the profile of metabolites with respect to methyl or ring oxidation in the presence of the fiber. He concluded that there was evidence in the literature that indicated that pretreatment of cultured cells with fibers can induce drug-metabolizing enzymes. He did not feel, however, that there was evidence that pretreatment actually upsets the outcome of the metabolism.

During the session on the methods of control and analysis of asbestos in water, J. Long (U.S. EPA) described the status of analysis of asbestos. He cited several publications which are or will be available. The most current EPA method for asbestos in water is the "Interim Method for Determining Asbestos in Water" by C. H. Anderson and J. M. Long (Publ. No. EPA-600/4-80-005). A new EPA-sponsored publication entitled "Development of Improved Analytical Techniques for Determination of Asbestos in Water Samples" by E. J. Chatfield, M. J. Dillion, and W. R. Stott is currently under review and expected to be out this year. A report entitled "A Rapid Screening Technique for Detection of Asbestos Fibers in Water Samples" by E. J. Chatfield and P. Riis is also in the final stages of review. Earlier EPA-sponsored work on a rapid method was reported in a 1978 publication entitled "Development of a Rapid Analytical Method for Determining Asbestos in Water" by C. W. Melton, J. J. Anderson, C. F. Dye, W. E. Chase, and R. E. Helffelfinger, (Pub. No. EPA-600/4-78-066).

S. Hayward asked Dr. E. Chatfield (Ontario Research Foundation) if plaity serpentine minerals interferred with asbestos determinations in his magnetic fiber alignment detector. Dr. Chatfield replied that one of the features of magnetic alignment is that the fibers are the only things which actually line up. Other types of particulates can be split into two different groups—one which is affected by the field and one which is not. Those which are not affected by the magnetic field and remain stable during the magnetic rotation, increase the general background under the peaks. Those that do rotate with the field for some reason, produce peaks centered on 45° and 25°. Therefore, one has to resort to a peak extraction routine in which the profile is disassembled into the two component peaks. Dr. Chatfield concluded that this can be done very readily and the asbestos or the fibrous component of the water sample can easily distinguished, even if there are 500 other types of particulates present. Dr. Chatfield estimated that the cost of a magnetic alignment system for rapid screening of asbestos samples would be between $30,000 and $50,000.

S. Hayward asked Dr. Logsdon (U.S. EPA) if he thought that it might be useful to add turbidity to a water supply so as to improve coagulation in those cases where there were a large number of fibers and not a lot of other particulates in the water. He described some cases in California where there was high asbestos concentrations in the water but nothing else. Dr. Logsdon replied that adding turbidity had been done in one experiment in Duluth by adding some clay, but it did not seem to be very promising. He commented further that it was not so much putting it in as it was telling the water utility operator, "I know that your water is clear, but it has to be superclear when it comes out of your filter. Good is not good enough, excellent is what you must strive for. In other words, you should have 0.1 NTU (turbidity units) instead of just meeting the drinking water regulation of 1 NTU."

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Announcement

Second International Symposium on Nephrotoxicity Guildford, England, August 6–9, 1984

The Second International Symposium on Nephrotoxicity will be held August 6–9, 1984, in Guildford, England, in conjunction with the Ninth IUPHAR Congress in London. The two major themes of the symposium will be: recent advances in monitoring the early renal functional changes in animals and man following mephrotoxic insult and the biological basis of chemically induced site-specific damage to the nephron.

These themes will attempt to cover several important areas relating to: (1) the role of renal cellular heterogeneity (both morphological and functional) in the primary pathogenesis and secondary consequences of chemically induced nephropathies; (2) improved morphological and biochemical methods for identifying early changes in the development of chemical related nephrotoxicity; (3) the effects of interactions between two or more chemicals in the development of toxic nephropathies; and (4) interspecies variability in the development and course of chemically induced renal damage, with special reference to extrapolating data to man.

The symposium will be multidisciplinary and consist of a small number of keynote lectures and as many unsolicited free communications as possible. The abstracts of papers presented at the symposium and the full proceedings will be published.

An integral part of the symposium will be a series of workshops which will concentrate on those techniques which can be used to study renal structure and function in health and in disease. These will take the form of discussions, live demonstrations and/or film-video presentations.

The closing date for registration is May 1, 1984. For further information, contact: Dr. P. H. Bach, Robens Institute of Health and Safety, University of Surrey, Guildford, GU2 5XH, Surrey, England.

Published Volumes of EHP

Volume no.	Subject	Supply
1	Polychlorinated Biphenyls	Out of Stock
2	Review – Perspective Articles	Out of Stock
3	Phthalate Esters	Out of Stock
4	Review – Perspective Articles	Out of Stock
5	Chlorinated Dibenzodioxins and Dibenzofurans	Available
6	Workshop on the Evaluation of Chemical Mutagenicity Data in Relation to Population Risk	Available
7	Low Level Lead Toxicity and the Environmental Impact of Cadmium	Available
8	Review – Perspective Articles	Available
9	Asbestos	Available
10	Mobile Air Emission, Biometeorological Hazards Abstracts on Heavy Metals in the Environment, Conference II	Available
11	Components of Plastics Manufacture	Available
12	Heavy Metals in the Environment	Available
13	US-USSR Environmental Health Conference	Available
14	Human Health Effects of New Approaches to Insect Pest Control	Available
15	Target Organ Toxicity: Liver and Kidney	Available
16	Target Organ Toxicity: Lung	Available
17	WHO/NIEHS Symposium on Plastics Manufacture	Available
18	Target Organ Toxicity: Development	Available
19	Environmental Arsenic and Lead	Available
20	Proceedings of the Second NIEHS Task Force NIEHS Science Seminar	Available
21	Vinyl Chloride Related Compounds	Available
22	Air Pollution and Human Health Extrapolation From Animal to Man	Available
23	Aspects of Polybrominated Biphenyls	Available
24	Target Organ Toxicity: Gonads PCBs	Available
25	Factors Influencing Metal Toxicity	Available
26	Target Organ Toxicity: Cardiovascular System Nervous System	Available
27	Higher Plants as Monitors of Environmental Mutagens Hazardous Solid Wastes and Their Disposal	Available
28	Cadmium	Available
29	Pollutants and High Risk Groups	Available
30	USA/USSR Cooperative Research	Available
31	Aneuploidy	Available
32	JAPAN/USA Biostatistics Statistics and the Environment	Available
33	Target Organ Toxicity: Intestines Effects of Increased Coal Utilization NIEHS Science Seminar	Available
34	Aquatic Toxicology Biological Effects of Mineral Fibers and Particulates	Available
35	Experimental Models for Pulmonary Research	Available
36	Application of Negative Ion Spectrometry	Available
37	Pollen Systems	Available
38	Target Organ Toxicity: Endocrine Systems	Available
39	Target Organ Toxicity: Blood	Available

Available – From NIEHS, P.O. Box 12233, Bld. 1, Rm. 107, Research Triangle Park, N.C. 27709. Subscription – From the Superintendent of Documents, US Government Printing Office, Washington, DC 20402 at a cost of $8.00 domestic and $10.00 foreign.

★ U.S. GOVERNMENT PRINTING OFFICE: 1983 O—735-036/2

Published Volumes of EHP (cont.)

Volume no.	Subject	Supply
40	Role of Metals in Carcinogenesis	Available
41	Polyvinyl Chloride	Available
42	Environmental Epidemiology	Available
43	Target Organ Toxicity: Immune System	Available
44	Target Organ Toxicity: Eye, Ear and Other Special Senses	Available
	Author and Subject Indexes, Volumes 26-40	
45	Phthalate Esters	Available
46	Drinking Water Disinfectants	Available
47	Mutagenicity and Carcinogenicity of Air Pollutants	Available
48	Health Effects of Toxic Wastes	Available
49	*N*-Substituted Aryl Compounds	Available
50	Tumor Promotion	Available
51	*In Vitro* Effects of Mineral Dusts	Subscription
52	Environmental Epidemiology	Subscription
	Air Quality Control	
	NIEHS Science Seminar	
53	Asbestos	Subscription

Instructions to Authors

Environmental Health Perspectives (EHP) is published by the National Institute of Environmental Health Sciences. The objectives of *EHP* are to communicate research findings of environmental health significance and to inform the scientific community of potential health hazards that are associated with particular elements in the environment. *Environmental Health Perspectives* publishes conference and workshop proceedings, perspective statements on selected problem areas, toxicologic information summaries, overviews of areas on environmental health, and reviews on specific environmental problems and agents.

Articles will be selected by the Editors on the basis of quality and pertinence. Only manuscripts prepared in English will be considered. All unsolicited manuscripts will be reviewed by members of our Editorial Review Board; other manuscripts will be reviewed at the discretion of the Editors. Letters commenting upon articles in *EHP* or items of general interest will also be considered.

Submission of Manuscripts

Manuscripts are of two types: *full-length* and *brief reports*. Brief reports are usually fewer than 1000 words in length.

Manuscripts must be typewritten copy, double-spaced on 8 1/2 × 11 inch paper and must be submitted in triplicate including three copies of each figure in addition to one set of *original* photographs or drawings. Submit manuscripts to:

Assistant Managing Editor
ENVIRONMENTAL HEALTH PERSPECTIVES (EHP)
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

Organization of Manuscript

Organization of the manuscript depends upon the nature of presentation and is left to the authors' discretion. However, a running title, key words and an abstract must accompany each manuscript. Please use the following guidelines for preparing the various sections of your manuscript:

Title Page: List title, authors (first or second name spelled out in full), complete name of institution or laboratory where work was done, and affiliation of each author. Indicate author to whom galley proofs and reprints should be sent (including telephone number).

Running Head. In addition to the main title, provide a short title (not to exceed 60 characters and spaces) to be used as a running head. List this information on the title page.

Key Words: On a separate sheet, list 5-10 key words to be used for indexing purposes. Also list on this sheet the names of the authors and the title of the paper.

Abstract: Begin this section on a separate page. The abstract should be 200-250 words in length.

Acknowledgements: Begin this section on a separate page. Financial support and technical assistance should be listed in this section.

References: Begin this section on a separate page. References are to be numbered in order of citation in the text and should be cited in the text in parentheses. The style for references to journals or books in the bibliography is as follows:

Journal Article

Viola, P. L., Biogotti, A., and Caputo, A. Oncogenic response to rat skin, lungs and bones to vinyl chloride. Cancer Res. 31: 516-522 (1971).

Book Chapter

Lohman, A. H. M., and Lammers, A. C. On the structure and fiber connections of the olfactory centres in mammals. In: Progress in Brain Research: Sensory Mechanisms, Vol. 23 (Y. Zotterman, Ed.), Elsevier, New York, 1967, pp. 65-82.

Book

Harper, R., Smith, E. C. B., and Land, D. B. Odour Description and Classification. American Elsevier, New York, 1968.

Editor as Author

Doty, R. L., Ed. Mammalian Olfaction, Reproductive Processes, and Behavior. Academic Press, New York, 1976, p. 337.

Abbreviate journal names according to *Chemical Abstracts* or *Biological Abstracts List of Serials (BIOSIS)*. List all authors; do not use et al. in the bibliography. Include title of journal article or book chapter and complete pagination. Reference to papers that have been accepted for publication but have not yet been published should be cited in the same manner as other references, with the name of the journal followed by "in press." "Personal Communication" and "Unpublished Observations" should *not* be listed in the bibliography. They are to be inserted at appropriate places in the text, in parentheses, without a reference number.

Footnotes: Footnotes should be avoided whenever possible. However, when they are necessary, please use the following guidelines: Use unnumbered footnotes to the title page to indicate author affiliations and addresses. Place these footnotes on the title page. Footnotes in text should be numbered in sequence, using superscript numbers. List these numbered footnotes on a separate page following references.

Tables: Each table should be on a separate page. Tables should be numbered with arabic numerals. The title should be centered and only the first word and proper nouns capitalized. Footnotes to the table should be indicated by lower case superscript letters, beginning with a for each table. Footnotes should be typed directly after the table.

Figures: Graphs, chemical structures and figures should be submitted either as original drawings in black India ink or as glossy photographic prints. Graphs and other drawn figures will be reproduced as submitted and will not be redrawn. One set of *original* unmounted glossy photographic prints or line drawings must accompany the submitted manuscript. In addition, one complete set (copies) of the figures should be attached to each copy of the manuscript. The style of figures should be uniform throughout the paper. Letters, numbers and symbols must be drawn to be at least 1.5 mm high after reduction. Choose a scale so that each figure may be reduced to two column width or one column width. Identify all figures on the back with authors' names and figure number and indicate TOP.

Figure Legends: Figure legends should be typed on a page separate from the figure. Legends should be numbered with arabic numerals, followed by the title with only the first word and proper nouns capitalized. Explanatory material should be in paragraph form below the title.

Abbreviations: Abbreviations used in the text should be defined in the text in parentheses when first mentioned. Nomenclature and symbols should conform to the recommendations of the American Chemical Society or the International Union of Pure and Applied Chemistry (IUPAC).

Galley Proofs, Reprints and Subscriptions

Galley Proofs and Reprints: Galley proofs and reprints will be mailed to the first author or the author designated on the title page. Once the author has received galley proofs, they must be mailed to the EHP office within 48 hours. Following publication, 200 free reprints will be mailed to the author.

Subscription Information: The three most recently published issues and yearly subscriptions are available from the Superintendent of Documents, Government Printing Office, Washington, DC 20402. Yearly subscription: $40 in U.S. and $50 outside U.S. Single issue: $8 in U.S. and $10 outside U.S. Back issues are available free of charge from the Assistant Managing Editor, EHP, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709. Refer to "Published Volumes of EHP" in the most recent issue to determine which issues are available free of charge and which issues are available by subscription.