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FINAL DRAFT:

TECHNICAL SUPPORT DOCUMENT FOR A PROTOCOL TO ASSESS ASBESTOS-RELATED RISK

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ACRONYMS

AM	alveolar macrophages
AP	alkaline phophatase
ATSDR	American Toxic Substances Disease Registry
BAL	bronchio-alveolar lavage
BrdU	bromodeoxyuridine
CalEPA	California Environmental Protection Agency
CFE	colony-forming efficiency
CHO	Chinese hamster ovary
EDXA	energy dispersive X-ray analysis
EGFR	Epithelial Growth Factor Receptor
ERK	EGFR-regulated kinase
ESR	electron spin residence
FBP's	fibrin breakdown products
HAF	human amniotic fluid
HBE	human bronchiolar epithelial
HNE	human neutrophil elastase
HTE	hamster tracheal epithelial
IPF	idiopathic pulmonary fibrosis
IRIS	Integrated Risk Information System
KGF	kertinocyte growth factor
LDH	lipid dehydrogenase
LPS	lipopolysaccharide
MAPK	mitogen activated protein kinase
MI	midget impinger
MLE	maximum likelihood estimate
MMVF's	man-made vitreous fibers
MnSOD	manganese-containing superoxide
mpcf	millions of particles per cubic foot
mppcf	millions of dust particles per cubic foot
MSHA	Mine Safety and Health Administration
NAC	N-acetylcysteine
NHBE	normal human bronchioepithelial
NHIS	National Health Interview Survey
NIOSH	National Institute for Occupational Safety and Health
OSHA	Occupational Safety and Health Agency
PARS	poly-ADP-ribosyl transferase
PCM	phase contrast microscopy
PE	pulmonary epithelial
PMN	polymorphonucleocyte
RBC's	red blood cells
RCF	refractory ceramic fiber
RNS	reactive nitrogen species
ROS	reactive oxygen species
RPM	rat pleural mesothelial
RR	relative risk
SAED	selected area electron diffraction
SEM	scanning electron microscopy
SHE	Syrian Hamster Embryo
SMG	small mucous granule
SMRs	standardized mortality ratios
THE	tracheal epithelial cells
TEM	transmission electron microscopy
TGF-β	transforming growth factor beta
TNF-α	tumor necrosis factor alpha
uPA	urokinase-type plasminogen activator
uPAR	urokinase-type plasminogen activator
U.S. EPA	U.S. Environmental Protection Agency
VCAM-1	vascular cell adhesion molecule

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1.0 EXECUTIVE SUMMARY

The purpose of this report is to provide a foundation for completing a state-of-the-art-protocol to assess potential human-health risks associated with exposure to asbestos. Such a protocol is intended specifically for use in performing risk assessments at Superfund sites, although it may be applicable to a broad range of situations.

The current report is a revision to a version originally submitted on September 4, 2001 (Berman and Crump 2001), which was the subject of a peer-review consultation held in San Francisco on February 25–26, 2003. In general, the expert panel endorsed the overall approach to risk assessment proposed in this report, although they highlighted areas where controversies persist.

The current report incorporates the changes recommended by the peer review consultation panel to correct minor problems with internal consistency and the overall transparency of the discussion that are needed to improve readability. Although some of the research and analyses recommended by the peer consultation panel are not complete, it is anticipated that the current document can be distributed for broader review and comment. Thus, the recommended approach to risk assessment can be considered for use in the interim, while the additional research and analyses recommended by the expert panel are completed. At that point, a final revision of this document will be developed and it is expected to serve as a component of a broader effort by the U.S. Environmental Protection Agency (U.S. EPA) to revise the Agency's current approach for assessing asbestos-related risks.

The approach currently employed at the U.S. EPA to evaluate asbestos-related risks (IRIS 1988) is based primarily on a document completed in 1986 (U.S. EPA 1986) and has not been changed substantially in the past 15 years, despite substantial improvements in asbestos measurement techniques and in the understanding of the manner in which asbestos exposure contributes to disease. Therefore, this document provides an overview and evaluation of the more recent studies and presents proposed modifications to the protocol for assessing asbestos-related risks that can be justified based on the more recent work.

As reported in several recent technical meetings and reinforced by information gleaned from the literature, the following were identified as issues that need to be addressed to develop a protocol for evaluating asbestos-related risk:

- whether the exposure-response models currently in use by the U.S. EPA for describing the incidence of asbestos-related diseases adequately reflect the time- and exposure-dependence for the development of these diseases;

- whether different potencies need to be assigned to the different asbestos mineral types to adequately predict risk for the disease endpoints of interest;

- to the extent that different asbestos mineral types are assigned distinct potencies, whether the relative *in vivo* durability of different asbestos mineral types determines their relative potency;

- whether the set of minerals included in the current definition of asbestos adequately covers the range of minerals that potentially contribute to asbestos-related diseases;

- whether the analytical techniques and methods currently used for determining asbestos concentrations adequately capture the biologically relevant characteristics of asbestos (particularly with regard to the sizes of the structures counted using the various analytical methods) so that they can be used to support risk assessment; and

- whether reasonable confidence can be placed in the cross-study extrapolation of exposure-response relationships that are required to assess asbestos-related risks in new environments of interest.

These outstanding issues (and other related considerations) are addressed in this document to provide a foundation for proposing a new approach for assessing asbestos-related risks. Although the objective of this evaluation was to identify the single best procedure, when current knowledge is inadequate for distinguishing among alternatives, options are presented along with a discussion of their relative advantages and limitations. In a few cases, limited and focused additional research studies are recommended, which may enhance the current state of knowledge sufficiently to resolve one or more of the important, remaining issues.

Background

Inhalation of asbestos dusts has been linked to several adverse health effects including primarily asbestosis, lung cancer, and mesothelioma (U.S. EPA 1986). Asbestosis, a chronic, degenerative lung disease, has been documented among asbestos workers from a wide variety of industries. Although asbestosis cases have been observed at some locations of current interest to the U.S. EPA, the disease is generally expected to be associated only with the higher levels of exposure commonly found in workplace settings and is not expected to contribute substantially to potential risks associated with environmental asbestos exposure. Therefore, asbestosis is only considered in this document to the extent required to address its putative association with lung cancer. Overall, the majority of evidence indicates that lung cancer and mesothelioma are the most important risks associated with exposure to low levels of asbestos.

The Asbestos Literature

A variety of human, animal, and tissue studies have provided insight into the nature of the relationship between asbestos exposure and disease. Ideally, human epidemiology studies are employed to determine the quantitative exposure-response relationships and the attendant risk coefficients for asbestos exposure. Exposure-response coefficients have been estimated for asbestos from approximately 20 epidemiology studies for which adequate exposure-response data exist. Such coefficients vary widely, however, and the observed variation has not been reconciled. Among the objectives of this study is to evaluate and account for the sources of uncertainty that contribute to the variation among the exposure-response coefficients derived from the literature so that these estimates can be reasonably interpreted and recommendations for their use in risk assessment developed.

Animal and tissue studies indicate that asbestos potency is a complex function of several characteristics of asbestos dusts including fiber size and fiber type (i.e., fiber mineralogy). Moreover, the influence of fiber size is a complex function of both diameter and length. Therefore, whenever the goal is to compare across samples with differing characteristics, it is not sufficient to report asbestos concentrations simply as a function of mass (or any other single measure), which is in stark contrast to the treatment of chemical toxins. It has generally been difficult to distinguish among the effects of fiber size and type in many studies because such effects are confounded and the materials studied have not been adequately characterized.

The Epidemiology Studies

The existing epidemiology studies provide the most appropriate data from which to determine the relationship between asbestos exposure and response in humans. As previously indicated, however, due to a variety of methodological limitations, the ability to compare and contrast results across studies needs to be evaluated to determine the confidence with which results from existing epidemiology studies may be extrapolated to new environments where risk needs to be assessed. This requires both that the uncertainties contributed by such methodological limitations and that several ancillary issues be addressed.

Briefly, the major kinds of limitations that potentially contribute to uncertainty in the available epidemiology studies include:

- limitations in air measurements and other data available for characterizing historical exposures;
- limitations in the manner that the character of exposure (i.e., the mineralogical types of fibers and the range and distribution of fiber dimensions) was delineated;
- limitations in the accuracy of mortality determinations or incompleteness in the extent of tracing of cohort members;
- limitations in the adequacy of the match between cohort subjects and the selected control population; and
- inadequate characterization of confounding factors, such as smoking histories for individual workers.

The existing asbestos epidemiology database consists of approximately 150 studies of which approximately 35 contain exposure data sufficient to derive quantitative exposure/response relationships. A detailed evaluation of 20 of the most recent of these studies, which includes the most recent follow-up for all of the cohorts evaluated in the 35 studies, was completed. The following conclusions result from this evaluation:

(1) To study the characteristics of asbestos that relate to risk, it is necessary to combine results (i.e., in a meta analysis) from studies of environments having asbestos dusts of differing characteristics. More robust conclusions regarding risk

can be drawn from an analysis of the set of epidemiology studies taken as a whole than results derived from individual studies.

(2) By adjusting for fiber size and fiber type, the existing database of studies can be reconciled adequately to reasonably support risk assessment.

(3) The U.S. EPA models for lung cancer and mesothelioma both appear to track the time-dependence of disease at long times following cessation of exposure. However, the relationship between exposure concentration and response may not be adequately described by the current models for either disease. There is some evidence that these relationships are supra-linear.

(4) Whereas the U.S. EPA model for lung cancer assumes a multiplicative relationship between smoking and asbestos, the current evidence suggests that the relationship is less than multiplicative, but possibly more complex than additive. However, even if the smoking-asbestos interaction is not multiplicative as predicted by the U.S. EPA model, exposure-response coefficients estimated from the model are still likely to relate to risk approximately proportionally and, consequently, may be used to determine an exposure index that reconciles asbestos potencies in different environments. However, adjustments to the coefficients may be required in order to use them to estimate absolute lung cancer risk for differing amounts of smoking. This issue needs to be investigated further in the next draft of this document.

(5) The optimal exposure index that best reconciles the published literature assigns equal potency to fibers longer than 10 μm and thinner than 0.4 μm and assigns no potency to fibers of other dimensions.

(6) The optimal exposure index also assigns different exposure-response coefficients for chrysotile and amphibole both for lung cancer and mesothelioma. For lung cancer the best estimate of the coefficient (potency) for chrysotile is 0.27 times that for amphibole, although the possibility that chrysotile and amphibole are equally potent cannot be ruled out. For mesothelioma the best estimate of the coefficient (potency) for chrysotile is only 0.0013 times that for amphibole and the possibility that pure chrysotile is non-potent for causing mesothelioma cannot be ruled out by the epidemiology data.

(7) Using the approach recommended in the U.S. EPA (1986) update, the lung cancer exposure-response coefficients (K_L values) estimated from 15 studies vary by a factor of 72 and these values are mutually inconsistent (based on non-overlap of uncertainty intervals). Using the approach based on the optimal exposure index that is recommended herein, the overall variation in K_L values across these studies is reduced to a factor of 50.

(8) Using the approach recommended in the U.S. EPA (1986) update, the mesothelioma exposure-response coefficients (K_M values) estimated from 10 studies vary by a factor of 1,089 and these values are likewise mutually

inconsistent. Using the approach based on the optimal exposure index that is recommended herein, the overall variation in K_M values across these studies is reduced to a factor of 30.

(9) The exposure index and exposure-response coefficients embodied in the risk assessment approach developed in this report are more consistent with the literature than the current U.S. EPA approach. In particular, the current approach appears highly likely to seriously underestimate risk from amphiboles, while possibly overstating risk from chrysotile. Furthermore, most of the remaining uncertainties regarding the new proposed approach also apply to the current approach. Consequently, it is recommended that the proposed approach begin to be applied in assessment of asbestos risk on an interim basis, while further work, as recommended below, is conducted to further refine the approach.

(10) The residual inconsistency in both the lung cancer and mesothelioma potency values is primarily driven by those calculated from Quebec chrysotile miners and from South Carolina chrysotile textile workers. The difference in the lung cancer potency estimated between these studies has long been the subject of much attention. A detailed evaluation of the studies addressing this issue, the results of our analysis of the overall epidemiology literature, and implications from the broader literature, indicate that the most likely cause of the difference between these studies is the relative distribution of fiber sizes in the two environments. It is therefore likely that the variation between these studies can be further reduced by developing improved characterizations of the dusts that were present in each of these environments (relying on either archived samples, or newly generated samples using technologies similar to those used originally).

Recommendations for Risk Assessment

Although gaps in knowledge remain, a review of the literature addressing the health-related effects of asbestos (and related materials) provides a generally consistent picture of the relationship between asbestos exposure and the induction of disease (lung cancer and mesothelioma). Therefore, the general characteristics of asbestos exposure that drive the induction of cancer can be inferred from the existing studies and were applied to define appropriate procedures for evaluating asbestos-related risk.

Optimum values for exposure-response coefficients for lung cancer and mesothelioma were derived in this analysis and can be combined with appropriately defined exposure estimates as inputs for the U.S. EPA lung cancer and mesothelioma models (respectively) to assess risk. Although these values are optimized within the constraints of the current analysis and reduce the apparent variation across published studies substantially, the need to manage and minimize risk when developing a general approach for assessing risk, is also recognized. Thus, to reduce the chance of under-estimating risks, a conservative set of potency estimates were also developed and are also presented. To assess risk, depending on the specific application, either the best-estimate risk coefficients or the conservative estimates can be incorporated into procedures described herein for assessing asbestos-related risks.

Tables are also provided that present estimates of the additional risk of death from lung cancer, from mesothelioma, and from the two diseases combined that are attributable to lifetime, continuous exposure at an asbestos concentration of 0.0001 f/cm^3 (for fibrous structures longer than 10 μm and thinner than 0.4 μm) as determined using TEM recommended methods. The risk estimates in these tables can be combined with appropriately determined estimates of exposure to develop estimates of risk in environments of interest.

Recommendations for Limited, Further Study

The two major objectives identified for further study are:

(1) to evaluate a broader range of exposure-response models in fitting the observed relationship between asbestos exposure and lung cancer or mesothelioma, respectively. For lung cancer models, this would also include an attempt to better account for the interaction between asbestos exposure and smoking; and

(2) to develop the supporting data needed to define adjustments for exposure-response coefficients that will allow them to be used with an exposure index that more closely captures the criteria that determine biological activity. Among other things, this work should focus on obtaining data that would permit more complete reconciliation of the exposure-response coefficients derived for Quebec miners and South Carolina textile workers.

2.0 INTRODUCTION

The purpose of this report is to provide a foundation for completing a state-of-the-art-protocol to assess potential human-health risks associated with exposure to asbestos. Such a protocol is intended specifically for use in performing risk assessments at Superfund sites, although it may be applicable to a broad range of situations.

The current report is a revision to a version originally submitted on September 4, 2001 (Berman and Crump 2001), which (among other things) includes both an extensive review of the general literature and a detailed analysis of the existing epidemiology studies. These are reproduced in the current report in Chapter 6 and Chapter 7/Appendix A (respectively).

The September 4, 2001 version was also the subject of a peer-review consultation held in San Francisco on February 25–26, 2003. The comments of the expert panel convened to conduct the peer review are included in this report as Appendix B.

In general, the expert panel endorsed the overall approach to risk assessment proposed in this report, although they highlighted areas where controversies persist. They also suggested additional research and analyses to attempt to resolve some of the outstanding controversies and to refine several of the details of the approach. In addition, they offered recommendations for modifications to improve the overall transparency and readability of the earlier version of this report.

The current report incorporates the changes recommended by the peer review consultation panel to correct minor problems with internal consistency and the overall transparency of the discussion that are needed to improve readability. Although some of the research and analyses recommended by the peer consultation panel are not complete, it is anticipated that the current document can be distributed for broader review and comment. Thus, the recommended approach to risk assessment can be considered for use in the interim, while the additional research and analyses recommended by the expert panel are completed. At that point, a final revision of this document will be developed and it is expected to serve as a component of a broader effort by the U.S. Environmental Protection Agency (U.S. EPA) to revise the Agency's current approach for assessing asbestos-related risks.

The approach currently employed by U.S. EPA to evaluate asbestos-related risks (IRIS, 1988) is based primarily on a document completed in 1986 (U.S. EPA 1986) and has not changed in the past 15 years, despite substantial improvements in asbestos measurement techniques and in the understanding of the manner in which asbestos exposure contributes to disease. Therefore, among other things, this document provides an overview and evaluation of more recent studies and presents proposed modifications to the current approach for assessing asbestos-related risks that can be justified based on the more recent work.

In May 2001, the U.S. EPA along with the California Environmental Protection Agency (CalEPA), the National Institute for Occupational Safety and Health (NIOSH), the American Toxic Substances Disease Registry (ATSDR), and the Mine Safety and Health Administration (MSHA) hosted an international conference on asbestos in Oakland, California that was attended

by leading international experts on asbestos. The state of knowledge concerning such issues as the nature of asbestos, the measurement of asbestos, and the relationship between asbestos exposure and the induction of disease was reviewed during this conference. Particular emphasis was placed on identifying important knowledge gaps in these areas.

By coupling the outstanding issues identified at the Oakland meeting with additional information gleaned from the literature, the following set of issues was identified as risk-specific issues of current interest:

- whether the exposure-response models currently in use by the U.S. EPA for describing the incidence of asbestos-related diseases adequately reflect the time- and exposure-dependence for the development of these diseases;

- whether different potencies need to be assigned to the different asbestos mineral types to adequately predict risk for the disease end points of interest;

- to the extent that different asbestos mineral types are assigned distinct potencies, whether the relative *in vivo* durability of different asbestos mineral types determines their relative potency;

- whether the set of minerals included in the current definition of asbestos adequately covers the range of minerals that potentially contribute to asbestos-related diseases;

- whether the analytical techniques and methods currently used for determining asbestos concentrations adequately capture the biologically relevant characteristics of asbestos (particularly with regard to the sizes of the structures counted using the various analytical methods) so that they can be used to support risk assessment; and

- whether reasonable confidence can be placed in the cross-study extrapolation of exposure-response relationships that are required to assess asbestos-related risks in new environments of interest.

These outstanding issues (and other related considerations) are addressed in this document to provide a foundation for proposing a new approach for assessing asbestos-related risks. Compared to the current U.S. EPA approach, it is shown that the new approach better predicts risks among the environments in which asbestos-related risks have been previously evaluated (i.e., the published epidemiology studies) so that the new approach can be used to predict risks in unstudied environments of interest with greater confidence than predictions based on the current approach. Moreover, completing the additional research and analysis recommended by the expert panel (Appendix B) should facilitate further refinement while providing additional opportunities to better evaluate and validate the proposed approach.

The remainder of this document is divided into 6 chapters:

- Chapter 3 presents an overview of the general considerations that need to be addressed to assess asbestos-related risks (including considerations associated with the manner in which asbestos exposures are characterized, the manner in which risk is modeled from existing data, and the manner that risk models are then applied to estimate risk in new environments). The nature of the diseases commonly attributed to asbestos exposure are also briefly described;

- Chapter 4 presents a background discussion that addresses the definition of asbestos, the mineralogy of asbestos, the morphology of asbestos-containing dusts to which people are typically exposed, the capabilities and limitations of analytical techniques and methods used to determine airborne asbestos concentrations, and the structure and function of the human lung;

- Chapter 5 provides a description of the kinds of literature studies that are commonly used to support development of a protocol to assess risk, with particular emphasis on identifying their relative strengths and weaknesses;

- Chapter 6 presents a review of the literature with particular emphasis on studies published since the Health Effects Assessment Update (U.S. EPA 1986). Combined with a description of supporting analyses, the review is focused on reconciling apparently conflicting studies and hypotheses (when possible) and identifying the best candidate procedures for assessing asbestos-related risks. To reconcile studies, the strengths and weaknesses common to various types of studies are explicitly considered;

- Chapter 7 presents a reevaluation of the published epidemiology studies that, among other things, is designed to address and (when possible) resolve the outstanding issues of current interest; and

- Chapter 8 presents a proposed, new approach for assessing asbestos-related risks.

Regarding Chapter 8, although the objective of this document was to identify the single best procedure, when current knowledge is inadequate for distinguishing among alternatives, options are presented along with a discussion of their relative advantages and limitations. A few limited and focused additional research studies are recommended, which have the potential to enhance the current state of knowledge sufficiently to resolve one or more of the important, remaining issues. These recommended studies parallel those identified by the peer review panel (Appendix B).

This report is part of a series of documents developed as part of a multi-task project to develop a set of mutually consistent methods for determining asbestos concentrations in a manner useful for assessing risk and a companion protocol for conducting such risk assessments. A method for the determination of asbestos in air (Chatfield and Berman 1990) and a companion technical background document (Berman and Chatfield 1990) were published by the U.S. EPA in 1990. The air method has since been superceded (improved) by the ISO Method (ISO 1995). A

method for the determination of asbestos in soils and bulk materials (Berman and Kolk 1997) was also published by the U.S. EPA and the draft of an improved version was also recently completed (Berman and Kolk 2000). The recommendations in this document should serve as the basis for development of the companion risk-assessment protocol.

3.0 OVERVIEW

Inhalation of asbestos dusts has been linked to several adverse health effects including primarily asbestosis, lung cancer, and mesothelioma (U.S. EPA 1986). The kinds of lung cancers linked to asbestos exposure are similar to those induced by smoking and a greater-than-additive effect has been observed from combined exposure (see, for example, Liddell and Armstrong 2002). Mesothelioma is a rare cancer of the membranes that line the pleural cavity (containing the heart and lungs) and the peritoneal cavity (i.e., the gut). Although there is some evidence of a low background incidence of spontaneous mesotheliomas, this cancer has been associated almost exclusively with exposure to asbestos and certain other fibrous substances (HEI-AR, 1991).

Asbestosis, a chronic, degenerative lung disease, has been documented among asbestos workers from a wide variety of industries. Although asbestosis cases have been observed at some locations of current interest to the U.S. EPA, the disease is generally expected to be associated only with the higher levels of exposure commonly found in workplace settings and is not expected to contribute substantially to risks potentially associated with environmental asbestos exposure. Therefore, asbestosis is only considered in this document to the extent required to address its putative association with lung cancer. Overall, the majority of evidence indicates that lung cancer and mesothelioma are the most important risks associated with exposure to low levels of asbestos.

> The primary route of exposure of concern in association with asbestos is inhalation. There is little evidence that ingestion of asbestos induces disease (see, for example, IRIS 1988; U.S. EPA 1986). Therefore, this study is focused on inhalation hazards, and other routes of exposure are not addressed.

Gastrointestinal cancers and cancers of other organs (e.g., larynx, kidney, and ovaries) have also been linked with asbestos exposures (by inhalation) in some studies. However, such associations are not as compelling as those for lung cancer and mesothelioma and the potential risks from asbestos exposures associated with these other cancers are much lower (U.S. EPA 1986). Consequently, by addressing the more substantial asbestos-related risks associated with lung cancer and mesothelioma, the much more moderate risks potentially associated with cancers at other sites are also addressed by default. Therefore, this document is focused on the risks associated with lung cancer and mesothelioma.

A variety of human, animal, and tissue studies have provided insight into the nature of the relationship between asbestos exposure and disease. Ideally, human epidemiology studies are employed to determine the quantitative exposure/response relationships and the attendant risk coefficients for asbestos exposure. Risk coefficients have been estimated for asbestos from approximately 20 epidemiology studies for which adequate exposure-response data exist. However, such coefficients vary widely (for lung cancer, coefficients vary by more than a factor of 70 and, for mesothelioma, they vary by more than a factor of 1,000) and this variation has not been reconciled. Among the objectives of this study, one is to evaluate and account for the sources of uncertainty that contribute to the variation among the risk coefficients derived from the literature so that these estimates can be reasonably interpreted and recommendations for their use in risk assessment developed.

Animal and tissue studies indicate that asbestos potency is a complex function of several characteristics of asbestos dusts including fiber size and fiber type (i.e., fiber mineralogy). Moreover, the influence of fiber size is a complex function of both diameter and length. Therefore, whenever the goal is to compare across samples with differing characteristics, it is not sufficient to report asbestos concentrations simply as a function of mass (or any other single parameter), which is in stark contrast to the treatment of chemical toxins. It has generally been difficult to distinguish among the effects of fiber size and type in many studies because such effects are confounded and the materials studied have not been adequately characterized.

The influence of different characteristics of asbestos dusts upon risk cannot be adequately evaluated in the existing epidemiological studies because the analytical techniques used to monitor asbestos exposure in these studies are not capable of resolving all of the characteristics of asbestos dusts that other types of studies indicate are important. Moreover, the exposure indices (the range of structure sizes and shapes used to characterize an asbestos dust) that are employed in the existing epidemiology studies may not correspond with the characteristics of asbestos that best relate to biological activity. This hinders the ability to reconcile risk (exposure-response) coefficients derived from different studies. It also limits the confidence with which risk coefficients derived from the existing epidemiology studies can be applied to assess risks from asbestos exposure in other environments. Such limitations are explored in this report, along with potential remedies.

Based on the current approach for evaluating asbestos-related cancer risk (U.S. EPA 1986), risk is estimated as the product of a risk coefficient and a mathematical function that depends on the level of exposure, the duration of exposure, and time. The risk coefficient for lung cancer is generally denoted as, "K_L" and the one for mesothelioma as "K_M". A detailed description of both the current lung cancer and mesothelioma models is provided in Chapter 7. The models differ depending on whether lung cancer or mesothelioma is being considered.

For lung cancer, the model estimates *relative* risk, which means that the increase in lung cancer incidence that is attributable to asbestos exposure is proportional to the background lung cancer incidence in the exposed population. The background cancer incidence is the rate of lung cancer that would be expected to occur in the population in the absence of asbestos exposure. In other words, background lung cancer incidence is the lung cancer rate for the exposed population that is attributable to all causes other than asbestos.

Among the implications of the lung cancer model is that the combined effects of asbestos exposure and smoking is multiplicative and, until recently, the majority of studies have suggested such a multiplicative relationship (see, for example, Hammond et al. 1979). However, newer studies (for example, Liddell and Armstrong 2002) suggest a complex relationship that is closer to additive than multiplicative. Such considerations are addressed further in Chapter 7.

The current EPA model for mesothelioma is an *absolute* risk model. This means that the increase in mesothelioma risk attributable to asbestos is independent of the background rate of mesothelioma, which is negligible in the general population.

Ideally, the risk coefficients derived from the existing epidemiology studies can be combined with measurements from other exposure settings to estimate lung cancer and mesothelioma risks in these other exposure settings. However, such risk estimates are only valid if both of the following conditions are met:

(1) asbestos is measured in the exposure setting of interest in the identical manner in which it was measured in the study from which the corresponding risk coefficients are derived; and

(2) such measurements reflect the characteristics of asbestos exposures that determine risk.

A growing body of evidence indicates that the way in which asbestos concentrations were measured in the existing epidemiology studies do not reflect the characteristics of asbestos exposure that determine risk. Therefore, measuring asbestos concentrations in the same way in exposure settings of interest may not be sufficient to assure the validity of risk estimates derived using the published risk coefficients (and the corresponding models). This is because the second of the above-listed conditions would not be satisfied.

Considerations necessary to compare risk coefficients derived in different exposure settings (or to apply a coefficient to predict risk in a setting different from the one in which the coefficient was derived) have been elucidated clearly in a mathematical model (Chesson et al. 1990). The consequences of the model indicate that adjusting the existing risk coefficients so that they reflect asbestos characteristics that determine biological activity requires knowledge of the fiber size distributions of the dusts studied in the *original* epidemiology studies. To the extent they exist, such data may be used to normalize each of the published risk coefficients so that they relate to a common exposure index reflecting asbestos characteristics that determine biological activity.

Among the goals of this evaluation is to explore the possibility of defining an improved exposure index (that better reflects biological activity) and to use this index to reconcile the epidemiology data (see Chapter 7). We also evaluated improved ways of simultaneously accounting for the effects of both fiber size and type.

Unfortunately, some of the issues that need to be resolved to support development of a protocol for assessing asbestos-related risks cannot be entirely resolved with existing data. Therefore, in later chapters (i.e., Chapters 6, 7, and 8), we have attempted to identify such issues, to assess their relative importance, and, when deemed appropriate, to propose limited and focused research projects designed to provide the data required to reduce the impacts of such knowledge gaps.

4.0 BACKGROUND

Asbestos is a term used to describe the fibrous habit of a family of hydrated metal silicate minerals. The most widely accepted definition of asbestos includes the fibrous habits of six of these minerals (IARC 1977). The most common type of asbestos is chrysotile, which is the fibrous habit of the mineral serpentine, a magnesium silicate. The other five asbestos minerals are all amphiboles (i.e., all partially hydrolyzed, mixed-metal silicates). These are: fibrous reibeckite (crocidolite), fibrous grunerite (amosite), anthophyllite asbestos, tremolite asbestos, and actinolite asbestos.

All six of the minerals whose fibrous habits are termed asbestos occur most commonly in non-fibrous, massive habits. While unique names have been assigned to the asbestiform varieties of three of the six minerals (noted parenthetically above) to distinguish them from their massive forms, such nomenclature has not been developed for anthophyllite, tremolite, or actinolite. Therefore, when discussing these latter three minerals, it is important to specify whether a massive habit of the mineral or the fibrous (asbestiform) habit is intended.

Although other minerals may also occur in a fibrous habit, they are not generally included in the definition of asbestos either because they do not exhibit properties typically ascribed to asbestos (e.g., high tensile strength, the ability to be woven, heat stability, and resistence to attack by acid or alkali) or because they do not occur in sufficient quantities to be exploited commercially.

The first four of the six asbestos minerals listed above have been exploited commercially (IARC 1977). Of these, chrysotile alone accounts for more than 90% of the asbestos found in commercial products.

Importantly, it is neither clear whether the term asbestos maps reasonably onto the range of fibrous minerals that can contribute to asbestos-like health effects nor whether individual structures of the requisite mineralogy must formally be asbestiform to contribute to such health effects.

Regarding whether the term asbestos is a useful discriminator for health effects, it is well established that erionite (a fibrous zeolite not related to asbestos) is a potent inducer of mesothelioma (Baris et al. 1987), which is one of the two primary asbestos-induced cancers (see Chapter 3). It is therefore possible that the fibrous habits of at least some other minerals not formally included in the current definition of asbestos may contribute to the induction of asbestos-related diseases. Therefore, an efficient procedure is needed for separating potentially hazardous materials from those that are most likely benign.

There are two issues related to the question of whether fibers must formally be asbestiform to contribute to health effects. The first involves the relationship between fiber structure and disease induction and the second involves measurement. Although the evidence is overwhelming that the size and shape of a fiber affects the degree to which it contributes to the induction of disease (this is addressed in detail in Chapter 6), it does not appear that sizes inducing biological activity are well distinguished by criteria that define the asbestiform habit. Therefore, depending on the definition employed for the fibers (or fibrous structures) that are

counted during an analysis, it may or may not be necessary to distinguish formally between asbestiform and non-asbestiform structures for the concentrations derived from such a count to adequately reflect biological activity.

The dimensions of an asbestiform fiber are determined by the manner in which the fiber grows (Addison 2001). In contrast, the massive forms of various minerals, when cleaved, also form elongated particles (termed "cleavage fragments") and, depending on the definition employed for fibrous structures during an analysis, such cleavage fragments may or may not be included along with asbestiform fibers in a count (see Section 4.3). Although it is clear from the manner in which they are each formed that the surface properties of asbestiform fibers and cleavage fragments are likely to be very different (for example, the latter will have many "unsatisified" chemical bonds), the degree to which such differences affect the toxic potency for comparable sized structures is not currently known.

Although it is beyond the scope of this document to present a detailed treatise on asbestos mineralogy, the morphology of asbestos dusts, or the nature and limitations of analytical techniques and methods used to determine asbestos concentrations, a brief overview of these topics is presented in the following sections both to identify issues that need to be addressed as part of the development of an appropriate protocol for assessing asbestos risks and to provide the background required to facilitate evaluation of the relevant issues. In that regard, a section on lung physiology and function is also provided.

4.1 THE MINERALOGY OF ASBESTOS

As previously indicated, the six asbestos minerals can be divided into two general classes. Chrysotile is the fibrous habit of the mineral serpentine (Hodgson 1965). The smallest fibrils of chrysotile occur as rolled sheets or hollow tubules of this magnesium silicate mineral. The larger fibers of chrysotile form as tightly packed bundles of the unit fibrils.

Chrysotile fibrils typically range from 20 nm to approximately 300 or 400 nm (0.02 to 0.3 or 0.4 μm) in diameter. Although slightly thicker fibrils may occasionally occur, at some point the curvature induced by the mismatch between the magnesium and silicon layers of the fibril becomes thermodynamically unstable, so that production of thicker fibrils is prohibited (Addison 2001).

Chrysotile bundles are held together primarily by Van der Waals forces and will readily disaggregate in aqueous solutions containing wetting agents (e.g., soap). They will also readily disaggregate in lung surfactant (Addision, 2001).

The general chemical composition of serpentine is reported as $Mg_3(Si_2O_5)(OH)_4$ (Hodgson 1965). However, the exact composition in any particular sample may vary somewhat from the general composition. For example, aluminum may occasionally replace silicon and iron, nickel, manganese, zinc or cobalt may occasionally replace magnesium in the crystal lattice of chrysotile (serpentine).

The five other common varieties of asbestos are all fibrous forms of amphibole minerals (Hodgson 1965). These are ferro-magnesium silicates of the general composition:

$$A_{2-3}B_5(Si,Al)_8O_{22}(OH)_2$$

where:

A = Mg, Fe, Ca, Na, or K; and
B = Mg, Fe, or Al.

Some of these elements may also be partially substituted by Mn, Cr, Li, Pb, Ti, or Zn.

The fibrous habits of the amphibole minerals tend to occur as extended chains of silica tetrahedra that are interconnected by bands of cations (Hodgson 1965). Each unit cell typically contains eight silica tetrahedra and the resulting fibers tend to be rhomboid in cross-section. Amphibole fibers are generally thicker than chrysotile fibrils and may typically range from approximately 100 nm to 700 or 800 nm in diameter (Addison 2001). Substantially thicker fibers have also been observed.

4.2 MORPHOLOGY OF ASBESTOS DUSTS

Structures comprising the fibrous habits of the asbestos minerals come in a variety of shapes and sizes. Not only do single, isolated fibers vary in length and thickness, but such fibers may be found combined with other fibers to form bundles (aggregates of closely packed fibers arranged in parallel) or clusters (aggregates of randomly oriented fibers) or combined with equant particles to form matrices (asbestos fibers embedded in non-asbestos materials). Consequently, dusts (even of one mineral variety) are complex mixtures of structures. For precise definitions of the types of fibrous structures typically found in asbestos dusts, see ISO (1995).

Detailed descriptions of the characteristics of dusts typically encountered at environmental and occupational asbestos sites have been reported in the literature and the following summary is based on a previously published review (Berman and Chatfield 1990). Typically, the major components of the dust observed in most environments are non-fibrous, isometric particles. Fibrous structures consistently represent only a minor fraction of total dust. Asbestos structures represent a subset of the fibrous structures.

The magnitude of the fraction of total dust represented by fibers and the fraction of fibers composed of asbestos minerals vary from site to site. However, the fraction of asbestos in total dusts has been quantified only in a very limited number of occupational and environmental settings (see, for example, Cherrie et al. 1987 or Lynch et al. 1970).

The gross features of structure size distributions appear to be similar among asbestos dusts characterized to date (Berman and Chatfield 1990). The major asbestos fraction of all such dusts are small fibrous structures less than 5 μm in length. Length distributions generally exhibit a mode (maximum) between 0.8 and 1.5 μm with larger fibers occurring with decreasing frequency. Fibrous structures longer than 5 μm constitute no more than approximately 25% of total asbestos structures in any particular dust and generally constitute less than 10%.
In some environments, the diameters of asbestos fibers exhibit a narrow distribution that is

largely independent of length. In other environments, diameters appear to exhibit a narrow distribution about a mean for each specific length. In the latter case, both the mean and the spread of the diameter distribution increases as the length of the structures increase. The increase in diameter with length appears to be more pronounced for chrysotile than for the amphiboles, presumably due to an increase in the fraction of chrysotile bundles contributing to the overall distribution as length increases.

Only a few studies have been published that indicate the number of complex structures in asbestos size distributions. The limited data available indicate that complex structures may constitute a substantial fraction (up to one third) of total structures, at least for chrysotile dusts (see, for example, Sebastien et al. 1984). Similar results were also obtained during a re-analysis of dusts generated from the asbestos samples evaluated in the animal inhalation studies conducted by Davis et al. (Berman et al., in preparation). This is the same re-analysis used to support a study to identify asbestos characteristics that promote biological activity (Berman et al. 1995), which is discussed further in Section 6.4.3.

Historically, fibrous structures have been arbitrarily defined as structures exhibiting aspect ratios (the ratio of length to width) greater than 3:1 to distinguish them from isometric particles (Walton 1982). However, alternate definitions for fibers have also been proposed, which are believed to better relate to biological activity (see, for example, Berman et al. 1995 or Wylie et al. 1993). The degree to which fibers are combined within complex structures in a particular dust may also affect the biological activity of the dust (Berman et al. 1995). Therefore, proper characterization of asbestos exposure requires that the relative contributions from each of many components of exposure be simultaneously considered. Factors that need to be addressed include the distribution of structure sizes, shapes, and mineralogy in addition to the absolute concentration of structures. Such considerations are addressed further in Chapter 6. Thus, unlike the majority of other chemicals frequently monitored at hazardous wastes sites, asbestos exposures cannot be adequately characterized by a single concentration variable.

4.3 CAPABILITIES OF ANALYTICAL TECHNIQUES USED TO MONITOR ASBESTOS

Due to a complex history, a range of analytical techniques and methods have been employed to measure asbestos in the various studies conducted over time (Walton 1982). Use of these various methods has affected the comparability of results across the relevant asbestos studies (Berman and Chatfield 1990). Therefore, the relative capabilities and limitations of the most important methods used to measure asbestos are summarized here. Later sections of this report incorporate attempts to reconcile effects that are attributable to the limitations of the different methods employed in the various studies evaluated.

Analytical techniques used to measure airborne asbestos concentrations vary greatly in their ability to fully characterize asbestos exposure. The capabilities and limitations of four analytical techniques (midget impinger [MI], phase contrast microscopy [PCM], scanning electron microscopy [SEM], and transmission electron microscopy [TEM]) are described here. A general comparison of the relative capabilities and limitations of the analytical techniques introduced above is presented in Table 4-1.

Table 4-1. Capabilities and Limitations of Analytical Techniques Used for Asbestos Measurements[a]

Parameter	Midget Impinger	Phase Contrast Microscopy	Scanning Electron Microscopy	Transmission Electron Microscopy
Range of Magnification	100	400	2,000–10,000	5,000–20,000
Particles Counted	All	Fibrous Structures[b]	Fibrous Structures[b]	Fibrous Structures[b,c]
Minimum Diameter (size) Visible	1 μm	0.3 μm	0.1 μm	< 0.01 μm
Resolve Internal Structure	No	No	Maybe	Yes
Distinguish Mineralogy[d]	No	No	Yes	Yes

[a]The capabilities and limitations in this table are based primarily on the physical constraints of the indicated instrumentation. Differences attributable to the associated procedures and practices of methods in common use over the last 25 years are highlighted in Table 4-2.
[b]Fibrous structures are defined here as particles exhibiting aspect ratios (the ratio of length to width) greater than 3 (see Walton 1982).
[c]TEM counts frequently resolve individual fibrous structures within larger, complex structures. Based on internal structure, several different counting rules have been developed for handling complex structures. See the discussion of methods presented below.
[d]Most SEM and TEM instruments are equipped with the capability to record selected area electron diffraction (SAED) spectra and perform energy dispersive X-ray analysis (EDXA), which are used to distinguish the mineralogy of structures observed.

MI and PCM are the two analytical techniques used to derive exposure estimates in the majority of epidemiology studies from which the existing risk factors are derived. SEM is an analytical technique that has been employed in several key animal studies. TEM provokes interest because it is the only analytical technique that is potentially capable of distinguishing all of the characteristics of asbestos that potentially affect biological activity.

Although PCM was (and still is) widely used to characterize occupational exposures, its inability to distinguish between asbestos and non-asbestos and its lack of sensitivity limits its usefulness in environmental settings (Berman and Chatfield 1990). In fact, PCM analyses and TEM analyses showed no correlation among measurements collected during the cleanup of the 1991 Oakland Hills fire (Berman, unpublished data). Such lack of correlation is expected to be observed generally whenever measurements are collected at sites where asbestos concentrations are low enough that a substantial fraction of the structures counted by PCM are not asbestos. Consequently, TEM is the technique that has been recommended for use at Superfund sites (Berman and Kolk 1997; Chatfield and Berman 1990).

Importantly, the physical limitations of the various analytical techniques is only part of the problem. To provide reproducible results that can be compared meaningfully to other analyses in other studies, one must also consider the choice of procedures (methods) that address everything from sample collection and preparation to rules for counting and quantifying asbestos structures.

Table 4-2. Comparison of Applicable Methods For Measuring Asbestos in Air

	NIOSH 7400[a]	NIOSH 7402[b]	YAMATE[c]	AHERA[d]	ISO[e,f]
Analytical Technique	PCM	TEM	TEM	TEM	TEM
Preparation Methodology	Direct (no transfer)	Direct	Direct (Indirect Optional)	Direct	Direct (Indirect Optional)
Magnification	450x	10,000x	20,000x	15,000x - 20,000x	20,000x (total structures) 10,000x (structures longer than 5 µm)
Dimensions Counted					
Length (L):	L .> 5 µm	L > 1 µm	L > 0.06 µm	L > 0.5 µm	L > 0.5 µm, total structures L > 5 µm, long structures
Width (W):	W > 0.25 µm	3.0 > W > 0.04 µm	W > 0.02 µm	W > 0.02 µm	W < 3.0 µm (respirability)
Aspect Ratio (AR):	AR > 3	AR > 3	AR > 3	AR > 5	AR > 5
Sensitivity:					
s/cm^3				0.005	Adjustable
s/mm^2				70	10 for total structures 0.1 for long structures
Mineralogy Determined	No	Yes	Yes, except matrix particles	Yes, except matrix particles	Yes
Maximum Number Counted	100 structures	100 structures	100 structures	50 structures	100 total structures 100 long structures

Table 4-2. Comparison of Applicable Methods For Measuring Asbestos in Air *(continued)*

	NIOSH 7400[a]	NIOSH 7402[b]	YAMATE[c]	AHERA[d]	ISO[e,f]
Maximum Area Scanned	100 fields	100 grid openings	10 grid openings	Blanks: 10 openings Samples: 10 openings (assuming defined sensitivity is achieved with the collected air volumes).	Adjustable
Statistically Balanced Counting[g]	No	No	No	No	Yes
Counting Rules					
Structures	Count all structures exhibiting L > 5 µm, W < 3.0 µm, and AR > 3.	Count all structures exhibiting L > 1 µm, W < 3.0 µm, and AR > 3. Note PCME[h] fraction within count.	Count all structures exhibiting an AR > 3.	Count all structures with L > 0.5 µm exhibiting an AR > 5. Record individual fibers within all groupings with fewer than 3 intersections. Count structures with L > 5 µm separately (PCME[8]).	Count all structures with L > 0.5 µm or containing components with L > 0.5 µm that also exhibit an AR > 5. Separately identifiable components of parent structures that satisfy dimensional criteria are also separately enumerated. Conduct a similar count to that indicated above for structures with L > 5 µm.

Table 4-2. Comparison of Applicable Methods For Measuring Asbestos in Air *(continued)*

	NIOSH 7400[a]	NIOSH 7402[b]	YAMATE[c]	AHERA[d]	ISO[e,f]
Bundles	Bundles meeting overall dimensional criteria generally counted as single fibers unless up to 10 individual fiber ends can be distinguished within the bundle (representing 5 individual fibers).	Bundles meeting overall dimensional criteria generally counted as single fibers.	Bundles meeting overall dimensional criteria generally counted as single entities and noted as bundles on the count sheet.	Bundles of 3 or more fibers that meet the overall dimensional criteria are counted as single entities and noted as bundles on the count sheet.	Count parent bundles with L > 0.5 µm containing at least one component fiber that exhibits an AR > 5. Qualifying bundles that are components of other parent structures are also separately enumerated. For counts of structures with L > 5 µm, include only bundles longer than 5 µm.
Clusters	Within a cluster, count up to 10 individual fiber ends from (up to 5) fibers that meet the overall dimensional criteria. Otherwise, count a cluster as a single entity.	Within a cluster, count up to 3 individual fibers that meet the overall dimensional criteria. Otherwise, clusters that contain more than 3 fibers that meet the overall dimensional criteria are counted as single clusters.	Within a cluster, count up to 3 individual fibers that meet the overall dimensional criteria. Otherwise, clusters that contain more than 3 fibers that meet the overall dimensional criteria are counted as single clusters.	A collection of fibers with more than 2 intersections where at least one individual projection meets the overall dimensional criteria is counted as a single cluster.	Distinguish “disperse” and “compact” clusters. Count all clusters containing at least one component fiber or bundle satisfying appropriate dimensional criteria. Separately enumerate up to 10 component structures satisfying appropriate dimensional criteria.

Table 4-2. Comparison of Applicable Methods For Measuring Asbestos in Air *(continued)*

	NIOSH 7400[a]	NIOSH 7402[b]	YAMATE[c]	AHERA[d]	ISO[e,f]
Matrices	Count up to 5 fibers emanating from a clump (matrix). Each individual fiber must meet the dimensional criteria.	Count individually identifiable fibers within a matrix. Fibers must individually meet the dimensional criteria.	Count as a single matrix, all matrices with at least one protruding or embedded fiber that meets the dimensional criteria.	Count as a single matrix, all matrices with at least one protruding fiber such that the protruding section meets the dimensional criteria.	Distinguish "disperse" and "compact" matrices. Count all matrices containing at least one component fiber or bundle satisfying appropriate dimensional criteria. Separately enumerate up to 10 component structures satisfying appropriate dimensional criteria.

[a]National Institute for Occupational Safety and Health (1985). *Method for Determination of Asbestos in Air Using Positive Phase Contrast Microscopy.* NIOSH Method 7400. NIOSH, Cincinnati, Ohio, U.S.A.

[b]National Institute for Occupational Safety and Health (1986). *Method for Determination of Asbestos in Air Using Transmission Electron Microscopy.* NIOSH Method 7402. NIOSH, Cincinnati, Ohio, U.S.A.

[c]Yamate, G., Agarwal, S.C., and Gibbons, R.D. (1984). *Methodology for the Measurement of Airborne Asbestos by Electron Microscopy.* U.S. EPA Report No. 68-02-3266. U.S. Environmental Protection Agency, Washington, D.C., U.S.A.

[d]U.S. Environmental Protection Agency (1987). *Asbestos Hazard Emergency Response Act: Asbestos-Containing Materials in Schools.* Final Rule and Notice (Appendix A: AHERA Method). Federal Register, 40 CFR 763, Vol. 52, No. 2, pp. 41826-41903, October.

[e]Chatfield, E.J. (1995). *Ambient Air: Determination of Asbestos Fibres, Direct Transfer Transmission Electron Microscopy Procedure.* Submitted to the International Standards Organization: ISO/TC 10312.

[f]Note that the ISO Method is a successor to the Interim Superfund Method: Chatfield, E.J. and Berman, D.W. (1990). *Superfund Method for the Determination of Asbestos in Ambient Air. Part 1: Method Interim Version.*. Prepared for the U.S. Environmental Protection Agency, Office of Emergency and Remedial Response, Washington, D.C. EPA/540-2-90/005a. May.

[g]Statistically balanced counting is a procedure incorporated into some asbestos methods (e.g. the Superfund Methods and the ISO Methods) in which long structures (typically longer than 5 μm) are counted separately during a lower magnification scan than used to count total structures (which are predominantly short). This procedure assures that the relatively rare longer structures are enumerated with comparable precision to that of the shorter structures.

[h]PCME stands for phase contrast microscope equivalent and indicates the fraction of structures observed by transmission electron micrscopy that would also be visible by phase contrast microscopy.

Multiple methods have been published for use in conjunction with several of the analytical techniques mentioned above (particularly TEM). Such methods differ in the procedures incorporated for sample preparation and for the manner in which asbestos structures are counted. The sample preparation requirements, conditions of analysis, and structure counting rules for several of the most commonly employed methods are presented in Table 4-2 to illustrate how the choice of method can result in substantially different measurements (even on duplicate or split samples).

The second column of Table 4-2 describes the specifications of the PCM method currently mandated by the Occupational Safety and Health Agency (OSHA) for characterizing asbestos exposure in occupational settings. Although this method is in common use today, several alternate methods for counting fibrous structures by PCM have also been used historically. Therefore, PCM measurements reported in earlier studies (including the available epidemiology studies) may not be comparable to PCM results collected today.

The last four columns of Table 4-2 describe TEM methods that are in current use. Comparison across these methods indicates:

- the shortest lengths included in counts using these methods vary between 0.06 and 1 μm. Given that structures shorter than 1 μm represent a substantial fraction of total asbestos structures in almost any environment (Section 4.2), this difference alone contributes substantially to variation in measurement results across methods;

- the definitions and procedures for counting complex structures (i.e., bundles, clusters, and matrices) vary substantially across methods, which further contribute to variation in measurement results. For example, the ISO Method requires that component fibers of clusters and matrices be counted separately, if they can be readily distinguished. In contrast, clusters are counted as single structures under the AHERA Method; and

- although all of the methods listed incorporate sample preparation by a direct transfer process (in which the fibers are counted in their original position on the filter), several of the methods have also been paired with an optional indirect transfer process (which involves ashing the original air filter, mixing the residue in water, sonicating, and re-suspending the fibers on a new filter). Measurements derived from split samples that are prepared, respectively, by direct and indirect transfer, can vary by factors as large as several 100 (Berman and Chatfield 1990). Typically, counts of asbestos structures on samples prepared by an indirect transfer procedure are greater than those derived from directly prepared samples by factors of between 5 and 50.

Given the combined effects from the physical limitations of the various techniques employed to analyze for asbestos and the varying attributes of the methods developed to guide use of these techniques, the relative capabilities and limitations of asbestos measurements derived, respectively, from paired methods and techniques in common use can be summarized as follows:

- all four techniques are particle counting techniques;
- neither MI nor PCM are capable of distinguishing asbestos from non-asbestos (i.e., they are incapable of determining structure mineralogy);
- counting rules used in conjunction with MI do not distinguish isometric particles from fibers;
- counting rules used in conjunction with PCM limits counting to fibrous structures longer than 5 μm with aspect ratios greater than 3:1;
- the range of visibility associated with PCM limits counting to fibers thicker than approximately 0.3 μm;
- under conditions typically employed for asbestos analysis, the range of visibility associated with SEM limits counting to fibers thicker than approximately 0.1 μm, which is only marginally better than PCM;
- SEM is capable of distinguishing asbestos structures from non-asbestos structures;
- TEM is capable of resolving asbestos structures over their entire size range (down to thicknesses of 0.01 μm);
- TEM is capable of distinguishing the internal components of complex asbestos structures; and
- TEM is capable of distinguishing asbestos structures from non-asbestos structures.

More detailed treatments of the similarities and differences between asbestos techniques and methods can also be found in the literature (see, for example, Berman and Chatfield 1990).

Due to the differences indicated, measurements from a particular environment (even from duplicate samples) that are derived using different analytical techniques and methods can vary substantially and are not comparable. In fact, results can differ by two or three orders of magnitude (Berman and Chatfield 1990). More importantly, because the relative distributions of structure sizes and shapes vary from environment to environment, measurements derived using different analytical techniques and methods do not even remain proportional from one environment to the next. Therefore, the results from multiple asbestos studies can only be meaningfully compared if the effects that are attributable to use of differing analytical techniques and methods can be quantified and reconciled. Few of the existing studies, however, document analytical procedures in sufficient detail to reconstruct exactly what was done.

4.4 THE STRUCTURE AND FUNCTION OF THE HUMAN LUNG

4.4.1 Lung Structure

The lungs are the organs of the body in which gas exchange occurs to replenish the supply of oxygen and eliminate carbon dioxide. To reach the gas exchange regions of the lung, inhaled air (and any associated toxins) must first traverse the proximal conducting (non-respiratory) airways of the body and the lung including the nose (or mouth), pharynx, larynx, trachea, and the various branching bronchi of the lungs down to the smallest (non-respiratory) bronchioles. Air then enters the distal (respiratory) portion of the lung, where gas exchange occurs.

In humans, the respiratory portion of the lungs are composed of the respiratory or aveolarized bronchioles, the alveolar ducts, and the alveoli (or alveolar sacs). There are approximately $3x10^8$ alveoli in human lungs (about 20 per alveolar duct) with a cumulative volume of $3.9x10^3$ cm^3 (Yeh and Harkema 1993). This represents approximately 65% of the total volume capacity of human lungs at full inspiration.

Yeh and Harkema (1993) also report that the ratio of lung volume to body weight is approximately constant across a broad range of mammals (from a shrew, 0.007 kg to a horse, 500 kg). Human lung volumes average a little more than 5 L.

Each human alveolus has a diameter of approximately 0.03 cm (300 μm) and a length of 0.025 cm (Yeh and Harkema 1993). The typical path length from the trachea to an alveolus is approximately 25 cm. The bronchi leading to each alveolus have branched an average of 16 times from the trachea (with a range of 9 to 22 branches). Importantly, the mean path length, the number of branches between trachea and alveolus, and the detailed architecture (branching pattern) of the respiratory region of the lung vary across mammalian species. For example, rats and mice lack respiratory bronchioles while macaque monkeys exhibit similar numbers of respiratory bronchiole generations as humans (Nikula et al. 1997). Furthermore, branching in humans tends to be symmetric (each daughter branch being approximately the same size and the angle of branching for each is similar but not quite equal) while rodents tend to exhibit monopodal branching in which smaller branches tend to come off at angles from a main trunk (Lippmann and Schlesinger 1984).

The gas exchange regions of the lung are contained within the lung parenchyma, which constitute approximately 82% of the total volume of the lungs (Gehr et al. 1993). Importantly, the lung parenchyma is not a "portion" of the lung; it fills virtually the entire volume of the organ traditionally visualized as the "whole" lung. Embedded within the parenchyma are the larger conducting airways of the lungs and the conducting blood vessels that transport blood to and from the capillaries that are associated with each alveolus. In the human lung, approximately 213 ml of blood are distributed over 143 m^2 of gas-exchange (alveolar) surface area (about the size of a tennis court). The gas-exchange surface area of lungs scale linearly with body weight over most mammalian species. The slope of the "reduced" line (where the Y-Axis is the ratio of the surface area to the surface area in a reference species and the X-Axis is the ratio of the body mass to the body mass of the same reference species) is 0.95. Figure 4-1 is a photomicrograph showing two views of a portion of lung parenchyma in the vicinity of a terminal bronchiole and an avleolar duct, which are labeled. The circular spaces in the left

portion of the figure and the cavities in the right portion of the figure are alveoli. Note the thinness of the walls (septa) separating alveoli.

Figure 4-1. The Structure of Lung Parenchyma Showing Alveoli and Alveolar Ducts (Source: St. George et al. 1993)

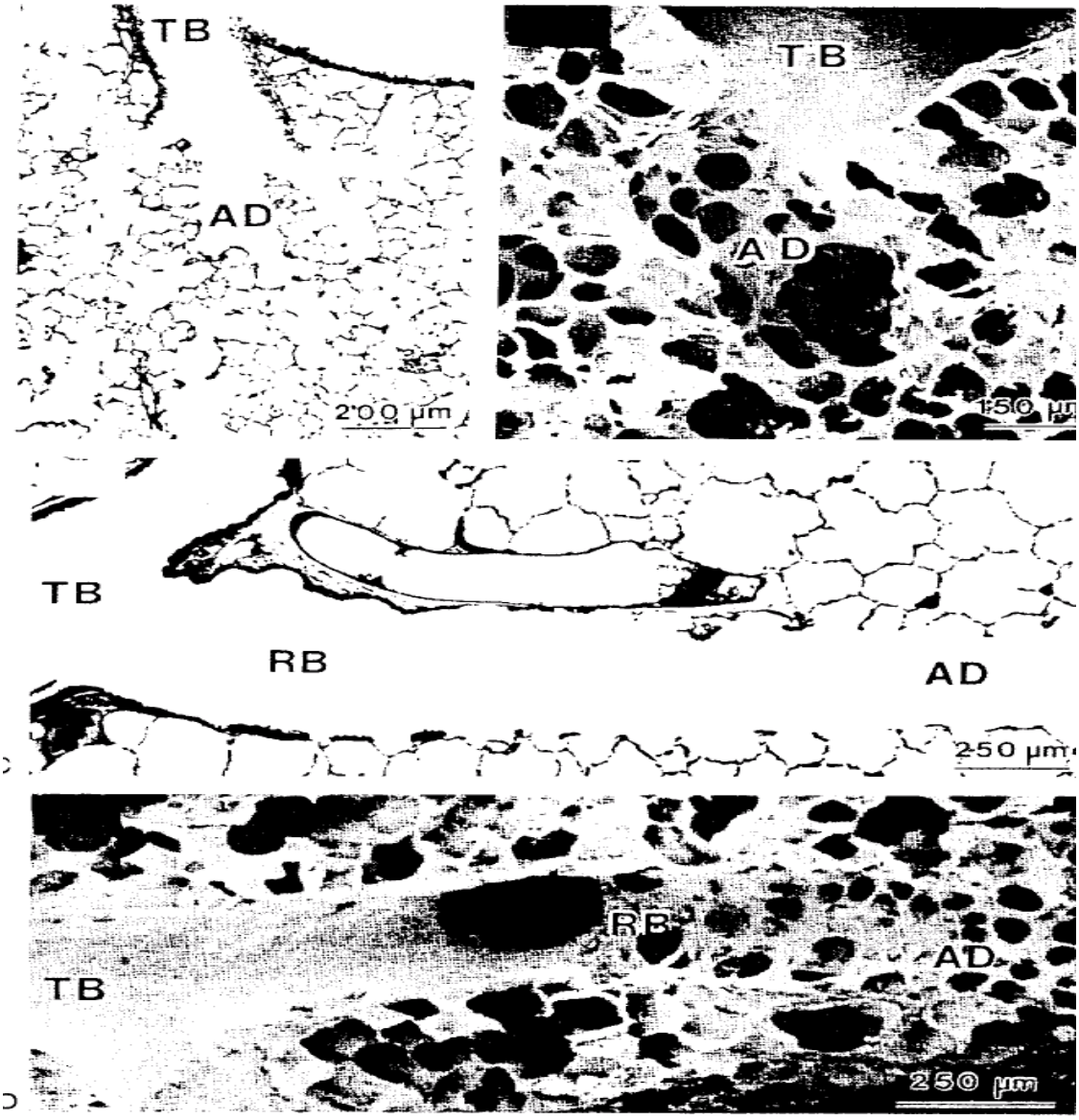

FIG. 10. LM and SEM comparison of the centriacinar region with two different organizations and **B**: The short or poorly developed respiratory bronchiole of the rat; **C** and **D**, the w alveolarized respiratory bronchiole of the cat. Terminal bronchiole (TB), respiratory bronchi (RB), and alveolar duct (AD). For details see ref. 106.

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Alveoli are separated from each other by alveolar septa that average only a few micrometers in thickness (Gehr et al. 1993). Gas-exchange capillaries run within these septa and the air-blood barrier, which averages only 0.62 μm in thickness, is composed of three layers: the alveolar epithelium, the interstitium, and endothelium. The epithelium is described in detail below. The interstitium is primarily composed of a collagenous, extracellular matrix, which constitute about two-thirds of the interstitial volume. There is also a collection of fibroblasts, macrophages, and other cells interspersed within the matrix (Miller et al. 1993). The cells of the interstitium constitute about one third of its volume. The endothelial layer is composed of the smooth muscle cells and connective tissue that constitute the walls of vascular capillaries. The amount of connective tissue in the septa between alveoli also varies between animal species; small rodents have less and primates more (Nikula et al. 1997).

Figures 4-2 and 4-3 show, respectively, a typical alveolar septum and a closeup of one portion of such a septum. In Figure 4-2, one can see that the septa themselves are thin and are filled almost entirely with capillaries. Figure 4-3 shows that the epithelial lining of an alveolus is no more than 1 μm thick, that the underlying interstitium is no thicker, and that the endothelium of the adjacent capillary is similarly thin. These three layers constitute the major tissues of the air-blood barrier (the rest of the barrier includes the limited quantity of blood plasma between the endothelial wall of a capillary and a red blood cell and the outer membrane of the red blood cell itself).

Figure 4-2. The Structure of the Inter-Alveolar Septa (Source: Gehr et al. 1993)

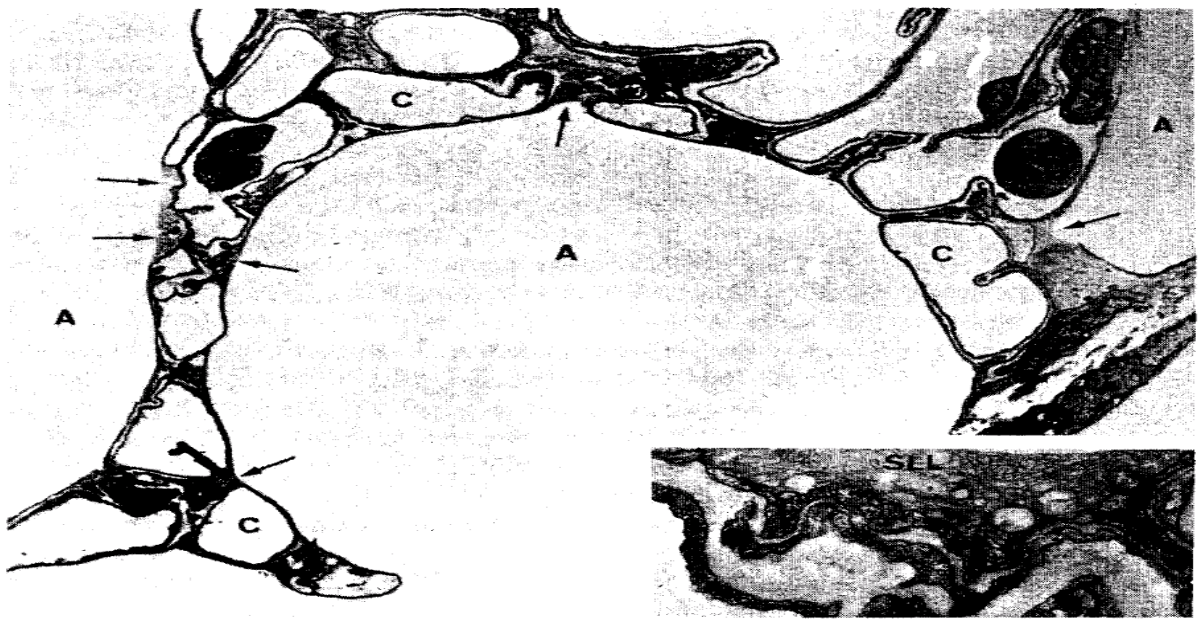

FIG. 10. Transmission electron micrograph of an alveolus from a dog lung fixed by intravascular perfusion. The lung was inflated with air at a pressure of 5 cm of water on the deflation limb of the last of three hysteresis cycles (approximately 60% TLC). It shows interalveolar septa that are folded at this degree of inflation. The surface lining layer (SLL) smoothes out every depression of the alveolar surface (*arrows*). A, alveoli; C, capillary. ×2,100. *Inset*: High power view of an epithelial depression filled with a fluid surface lining layer (SLL). ×14,600.

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Figure 4-3. The Detailed Structure of an Alveolar Wall that is Part of an Inter-Alveolar Septum (Source: Gehr et al. 1993)

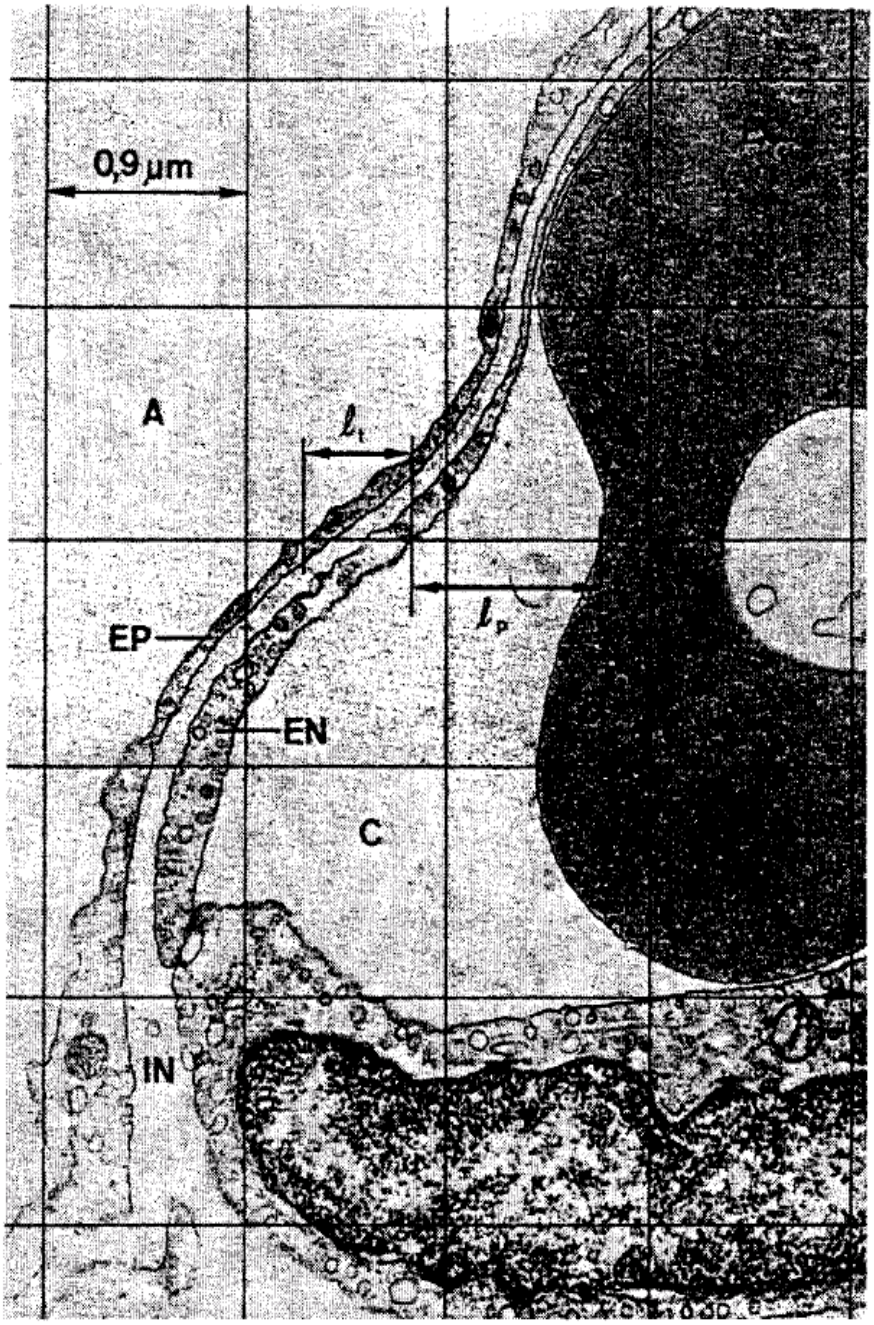

FIG. 19. Fraction of square lattice test grid superimposed on alveolar capillary to measure intercept lengths in tissue (l_t) and plasma (l_p) for the calculation of the harmonic mean barrier thickness. Note the erythrocyte (EC) in capillary (C) and that the barrier separating blood from alveolar air (A) is made of the three layers epithelium (EP), endothelium (EN), and interstitium (IN). ×29,000. (From ref. 80.)

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Gehr et al. (1993) also report that interalveolar septa constitute approximately 14% of the volume of the gas-exchange region of the lung (i.e., the lung parenchyma). Of this tissue mass, approximately 20% is endothelium, 55% is interstitium, and the rest is composed of cells associated with the alveolar epithelium. The remainder of the gas-exchange region is air space. Gehr et al. (1993) also report that the interalveolar septa fold to accommodate changes in lung volume during respiration, although the major contribution to lung volume changes (over the range of normal inspiration) appears to be the collapsing (folding) of alveolar ducts (Mercer and Crapo 1993).

According to Gehr et al. (1993), Type I epithelial cells, which constitute approximately 95% of the surface area of alveolar epithelium, are flat and platy (squamous), and average less than 1 μm in thickness (except where cell nuclei exist, which are approximately 7.5 μm in diameter and protrude into the alveolar space). Type II epithelial cells, which are cuboidal, constitute no more than 5% of the epithelial surface. Despite the small fraction of surface that they occupy, Type II cells serve to maintain the integrity of the overall epithelial lining so that, for example, they limit the tissue's permeability and control/prevent transport of macromolecules from the interstitium to the alveolar space, or the reverse (Leikauf and Driscoll 1993). Type II cells also secrete lung surfactant. The basement membrane of alveolar epithelium is a collagenous structure.

In contrast, the epithelial cells lining the trachea and bronchi (including the respiratory bronchioles) are ciliated and columnar and averages between 10 and 15 μm in thickness (based on photomicrographs presented in St. George et al. 1993). Tracheobronchial epithelium reportedly contains at least 8 distinct cell types (St. George et al. 1993): ciliated epithelium, basal cells (small flat cells situated along the basal lamina and not reaching the luminal surface), mucous goblet cells, serous cells, nonciliated bronchiolar (clara) cells, small mucous granule (SMG) cells, brush cells, and neuroendocrine cells. The relative abundance of the various cells varies across mammalian species as well as across the various airway generations (branches) and even the opposing sides of specific airways. The number of cells per length of basal lamina also varies across mammalian species.

4.4.2 The Structure of the Mesothelium

The mesothelium is a double layered membrane and each layer is a single-cell thick. The two layers of the mesothelium are separated by a space (e.g., the pleural space), which contains extracellular fluid and free macrophages (Kane and McDonald 1993). The pleural space is drained at fixed locations by lymphatic ducts. Each layer of the mesothelium overlies a collagenous basement membrane containing dispersed spindle cells. Depending on the location of the mesothelium, the basement membrane may overlie the skeletal muscle of the diaphragm or the rib cage (in the case of the parietal pleura, which is the outer layer). For the inner layer or visceral pleura, the basement membrane overlies visceral organs (including the lungs) within the rib cage. Healthy mesothelium is quiescent, meaning that cells are not actively dividing.

The relative size and thickness of mesothelial tissue varies across mammalian species (Nikula et al. 1997). For example, rats have relatively thin pleura with limited lymphatic ducts. In contrast, nonhuman primates have thicker pleura with greater lymphatic drainage than rats and humans have even thicker pleura and relatively abundant lymphatic ducts.

4.4.3 Cytology

Alveolar Epithelium. In the respiratory region of the lung, Type II epithelial cells are progenitor cells for Type I epithelium (Leikauf and Driscoll 1993). Type I epithelial cells are not proliferation competent (Nehls et al. 1997). After injury to Type I cells, Type II cells proliferate and reestablish the continuous epithelial surface. Type II cells also secrete surfactant. It appears that the identity and location of the progenitor cells for Type II epithelial cells are not currently known.

Injury or alteration of Type II cell function are associated with several diseases included idiopathic pulmonary fibrosis (Leikauf and Driscoll 1993). Also, crystalline silica and other toxic agents have been shown to directly modify Type II cellular activity. For example, crystalline silica (at sub-cytotoxic levels, <100 μg/ml) stimulates Type II proliferation in tissue culture. In contrast, neither titanium dioxide nor aluminum coated silica induce proliferation at corresponding concentrations.

In culture, Type II epithelial cells transform slowly into Type I cells and thus have limited population doubling capacity (Leikauf and Driscoll 1993). Rarely can the number of Type II cells expand past 3–10 passages (20–30 doublings). During this time, cells terminally differentiate, develop cross-linked envelopes, and appear squamous, enlarged, and multinucleated. The process is noted to be accelerated by the presence of transforming growth factor beta (TGF-β).

Macrophages. Alveolar macrophages (the largest population of macrophages in the lung) are mobile, avidly phagocytic, present antigens, and release cytokines that trigger various other immune responses (Leikauf and Driscoll 1993). They also initiate inflammatory responses and other repair mechanisms that are designed to restore tissue homeostasis.

The next largest population of macrophages in the lung are the interstitial macrophages (Leikauf and Driscoll 1993). These are localized in the peribronchial and perivascular spaces, the interstitial spaces of the lung parenchyma, the lymphatic channels, and the visceral pleura. The various populations of macrophages in the lung express different surface proteins, show different proliferative capacity, and show differences in metabolism.

The sizes of alveolar macrophages varies substantially across mammalian species (Krombach et al. 1997). Krombach and co-workers provide a table summary of the relative sizes across several species of interest:

Animal	Mean diameter (μm)	Mean Volume (μm^3)
Rats	13.1±0.2	1166±42
Syrian Golden Hamsters	13.6±0.4	1328±123
Cyanomolgus Monkeys	15.3±0.5	
Healthy Humans	21.2±0.3	4990±174

Note: as indicated later (Section 6.2), the relative size of various macrophages has direct implications regarding the dependence of clearance mechanisms on fiber size.

Mesothelium. Importantly, mesothelial cells are proliferation competent and may be their own progenitor cells (Kane and McDonald 1993). It is also possible, however, that as yet-to-be-identified progenitor cells are located along opposite walls of the pleura or at other locations within the pleural space.

Tracheo-bronchiolar epithelium. As previously indicated, tracheo-bronchiolar (i.e., non-respiratory) epithelium is composed primarily of ciliated, columnar cells that are 10 to 15 μm thick. Although some report that Clara cells serve as progenitor cells for tracheo-bronchiolar epithelium (Finkelstein et al. 1997), others report that both Clara cells and bronchiolar epithelium are proliferation competent (Nehls et al. 1997). It appears that the identity and location of the progenitor cells for Clara cells are not currently known.

4.4.4 Implications

The potential implications of the above observations concerning lung structure and cytology are:

- that the thicknesses of Type I epithelial cells, endothelial cells, and the interstitium in the alveolar septa are all very small relative to the lengths of the putative asbestos fibers that contribute to disease;
- that an entire alveolus is only 300 μm across and a typical Type I cell is 46 μm in radius by <1 μm thick;
- that distances across alveolar septa are only on the order of a few μm and such septa contain both the endothelium and the interstitum. Thus, the distances that have to be traversed to get to these structures are also small relative even to the length of a fiber;
- that the alveolar septa and the walls of the alveolar ducts fold during respiration, which may provide mechanical forces that facilitate movement of fibers into and through the alveolar epithelium;
- that Type I epithelium do not proliferate so they cannot be the cells that lead to cancer. It is the Type II epithelial cells (and potentially macrophages, basal cells, or endothelial cells) that contribute to cancer in the pulmonary portion of the lung. Type II cells eventually terminally differentiate to Type I cells;
- that other cells in the lung that have variously been reported to be proliferation competent (so that they potentially serve as target cells for the induction of cancer) include Clara cells and bronchiolar epithelial cells;
- that the distance from the most distal airways and alveoli to the pleura is small relative to the lengths of a fiber; and
- that mesothelial cells are proliferation competent and thus serve as potential targets for the induction of cancer.

5.0 THE ASBESTOS LITERATURE

This is a description of the common types of studies in the asbestos literature and an overview of the sources of potential uncertainty typically associated with each. Such limitations must be considered when drawing conclusions from these studies and, more importantly, when deriving inferences based on cross-study comparisons. Throughout this document, we have endeavored to identify the major sources of uncertainty in the studies we examined and have endeavored to account for such uncertainties during our evaluation and interpretation of study results.

The types of studies available for examining relationships between risk and asbestos exposure include human epidemiology studies, human pathology studies, a broad variety of animal studies, and a broad variety of *in vitro* studies in both tissue cultures and cell-free systems. To properly compare and contrast the results from such studies:

- the method(s) employed for asbestos characterization in each study need to be reconciled;
- the procedures employed for evaluating study endpoints need to be compared and contrasted;
- the relationship between the route of exposure employed in each type of study and the exposure route of interest (i.e., human inhalation) needs to be examined; and
- other major, study-specific sources of uncertainty need to identified and addressed.

Among study conditions and procedures that must be considered before evaluating study conclusions, it is particularly important to address the analytical methodologies employed to characterize the nature of exposures (or doses) in each study and such considerations are common to virtually all of the various types of studies of interest.

As indicated in Section 4.3, the only instrument capable of completely delineating asbestos structure-size distributions is TEM (or TEM combined with other techniques). Thus, for example, conclusions regarding variations in biological effects due to differences in such things as fiber size must be viewed with caution when fiber sizes are characterized using only PCM, SEM, or other, cruder analytical techniques. Similarly, the ability to adequately determine fiber mineralogy (fiber type), particularly of what may be minor constituents of various dusts or bulk materials, also depends strongly on the instrumentation employed for analysis as well as the strategy for sampling and for conducting the actual structure count. All of these factors must be considered.

Not only does the specific instrumentation (analytical technique) that is employed in an asbestos measurement affect the outcome of that measurement, but the particular method employed to guide the measurement affects the outcome. As previously indicated (Section 4.3), details concerning the definition of structures to be included in a count, the strategy for counting, and the minimum number of specific types of structures to be included in a count (all features that

vary across analytical methods) affect the precision with which fiber concentrations (particularly of longer and thinner fibers) are delineated. It is not uncommon, for example, that asbestos concentrations measured in the same sample may vary by several orders of magnitude, due simply to a difference in the analytical method employed for the analysis (even when the same analytical instrument is employed, see Section 4.3).

Other important sources of uncertainty tend to be study-type specific and are thus addressed separately below.

5.1 HUMAN EPIDEMIOLOGY STUDIES

A good overview of the kinds of limitations that contribute to uncertainty in the available epidemiology studies was presented in the Health Effects Assessment Update (U.S. EPA, 1986). As described in Appendix A of this document, while evaluating exposure-response factors derived from the human epidemiology studies, an attempt was made to address most of the major sources of uncertainty commonly associated with such studies, which are described briefly below.

Epidemiology studies, which track the incidence of disease (or mortality) within a defined group (cohort) sharing comparable exposures, have been performed on cohorts of workers exposed to asbestos and other mineral fibers in a variety of occupational and environmental settings. Among these, studies that include quantification of exposures are particularly useful for evaluating exposure-response relationships and deriving risk factors.

Generally, the most severe limitations in an epidemiology study involve the exposure data. Both estimates of the level of exposure and determination of the character of exposure are affected by such limitations. Regarding the character of exposure, because exposure measurements from most of the available quantitative epidemiology studies are based on MI measurements or PCM measurements, detailed characterization of the size distribution or the mineral type of fibrous structures (particularly of minor constituents) that contributed to exposure in such studies is generally lacking (Appendix A). This is particularly important because of the evidence that neither MI nor PCM are capable of providing measurements that remain proportional (across study environments) to the biologically-relevant characteristics of an asbestos dust (Berman et al. 1995). This limits the ability both to compare results across the existing studies and to extrapolate such results to new environments for which risks need to be estimated. At the same time, effects on the ability to observe exposure-response trends within a single study are not typically impaired.

Samples collected prior to the mid-1960s were often analyzed by measuring total dust in units of millions of particles per cubic foot (mpcf) using impingers or thermal precipitators. A description of the relative strengths and weaknesses of these techniques is provided in Section 4.3. The fibrous portion of the dust was not monitored. Impinger measurements are sometimes related to fiber counts (based on PCM) using side-by-side measurements of total dust and fiber counts collected during a relatively brief period of time (e.g., Dement et al. 1983a; McDonald et al. 1980b). However, the correlation between fiber counts and total dust is sometimes poor within a plant (i.e., a single study environment) and generally poor between plants (see, for example, U.S. EPA 1986). Thus, conversions based on limited sets of paired

measurements are of questionable validity. In some studies (e.g., McDonald et al. 1983b) the only available measurements are MI measurements (in mpcf) and these have been related to f/ml by PCM using conversion factors derived in other plants, which raises further questions concerning validity.

Even if all measurements could be adequately converted to PCM, this may still not be adequate for assessing risk in a manner that allows extrapolation across exposure environments or studies. Comparing exposure-response factors derived in different exposure environments (or extrapolating to new environments to predict risk) requires that asbestos measurements reflect the characteristics of asbestos structures (size, shape, mineralogy) that determine biological activity. If surrogate measures are employed (e.g., measures of asbestos structures displaying characteristics other than those that determine biological activity), there is no guarantee that concentrations of such surrogate measures and the true biologically active structures will remain proportional from one environment to the next. As a consequence, the relationship between exposure (measured by surrogate) and risk may not remain constant from one environment to the next. Importantly, several studies suggest that PCM may, at best, be no more than a surrogate measure (see, for example, Berman et al. 1995). Moreover, the technique was adapted to asbestos in an *ad hoc* fashion with only limited thought given to biological relevance (Walton 1982).

Use of surrogate measures of asbestos exposure may be less of a problem within a single exposure environment (where airborne asbestos structures likely have been generated in a similar manner from similar source material). Thus, surrogate measures of asbestos exposure may remain approximately proportional to the true biologically active structures, which suggests why monotonically increasing exposure-response relationships have likely been observed with PCM-measured concentrations *in single exposure environments.* In different exposure environments, however, the distribution of fiber sizes and types of airborne asbestos structures are likely different, since they are generated in different processes from different source material. There is thus little reason to expect surrogate measures of exposure to remain proportional across such environments.

None of the published epidemiology studies incorporate TEM measurements of asbestos and such measurements are not widely available in occupational settings (Appendix A). However, TEM is the method currently used (and recommended) to assess exposure in environmental settings, due both to questions concerning biological relevance (Berman et al. 1995 and addressed in detail in Chapter 6) and to problems with measuring environmental asbestos concentrations by PCM (Section 4.3).

In some cases, the limited exposure characterization presented in specific epidemiology studies can be augmented by pairing such studies with published TEM characterizations of dusts from the same or similar exposure settings, to the extent the appropriate supplemental studies are available. In fact, this is the procedure adopted in this document to adjust the existing risk factors to exposure indices that are thought to better relate to biological activity (described in detail in Section 7.4). Such an approach is limited, however, to the extent that the published asbestos characterizations actually represent exposure conditions in the corresponding epidemiology studies. To the extent reasonable, the limitations of this approach have been

addressed in this study by assigning and incorporating additional factors into the calculation of uncertainty intervals (defined in Appendix A) that are associated with the adjusted potency factors.

Regarding levels of exposure in the epidemiology studies, in most cases, air measurements were collected only infrequently and measurements may be entirely lacking from the earliest time periods, when exposures may have been greatest. In such cases, exposures are typically estimated either by extrapolation from periods when measurements are available or by expert judgement based on personal accounts and records of changes in plant operations, industrial hygiene procedures, air standards, etc. Moreover, the majority of exposure measurements used in these occupational studies are based on area (ambient) rather than personal samples. Typically, only a few areas of a plant have been sampled so that levels in other areas must be approximated using expert judgement by persons familiar with operations at the plant.

It is difficult to judge the degree that available asbestos concentration measurements are representative of actual exposures in the existing studies. In some cases, it seems likely that operations were shut down or otherwise modified in preparation for sampling. Likewise, in some operations there are brief episodes of very intense exposure and it is questionable whether such episodes are adequately represented in the available data.

Most of the asbestos measurements used in the published epidemiology studies were collected for insurance or compliance purposes. They were not intended to provide representative estimates of the direct level of exposure to workers. Some of the published epidemiology studies lack any direct exposure data. For example, exposures were estimated for the cohort studied by Seidman (1984) based on conditions simulated many years later in a similar plant to the one from which Seidman studied the original cohort. In fact, the equipment in the plant from which Seidman obtained exposure estimates came originally from the plant where Seidman studied the workers; it was purchased and moved. Recently, an epidemiology study was also completed for a cohort working at that new plant (Levin et al. 1998).

In addition to problems with the actual analysis of asbestos concentrations, individual exposures are generally estimated in the existing epidemiology studies by relating ambient asbestos measurements to job descriptions and integrating the duration of exposure over the recorded time that each worker spent in each job category. However, sometimes there are no records of specific areas in which an employee worked, so that work areas must be assumed based on job title. Some types of workers (e.g., maintenance workers) may have spent time in many different areas of a plant so their exposure varies from what might otherwise be assumed.

Although the greatest problems with the data in existing epidemiology studies likely lies within the estimates of exposure, problems with disease-response data also exist. Mesothelioma is rare and this disease may have been under-reported as a cause of death in older studies. This is probably less of a problem in more recent studies, since the association of mesothelioma with asbestos exposure is now well known. In fact, the opposite tendency (over-reporting) may now be occurring because of increased sensitivity by examiners (an asbestos worker with mesothelioma is now more likely to be eligible for compensation). Some studies have re-diagnosed causes of death from all of the available data (e.g., Selikoff et al. 1979); however,

this creates the problem of lack of comparability to control populations (for which such re-diagnosis is not generally performed).

The choice of an appropriate control population is also an important consideration. Local cancer rates may differ substantially from regional or national rates and the choice of an appropriate control is not always clear. A related problem is the lack of smoking data in many of the studies. Because of the interrelation between smoking and asbestos in lung cancer, errors could occur in lung cancer risk estimates if the smoking patterns of the cohort are substantially different from those of the control population.

In some of the studies, a substantial portion of the population is lost to follow-up (e.g., Armstrong 1988), and this adds additional uncertainty to the analysis. Also, the effect of exposure may be inaccurately evaluated if the follow-up of the population is too brief. This may be a limitation, for example, of the Levin et al. (1998) study.

Another problem frequently associated with these studies is that available data are not reported in a form that is well-suited to risk assessment. The EPA lung cancer model, for example, requires that exposure be estimated as cumulative exposure in f/ml·years excluding the most recent 10 years (U.S. EPA 1986, also described Section 7.2); generally the data are not published in this form. The data are also frequently not available in a form that permits study of the shape of the lung cancer exposure-response curve, so it is not possible to determine how well the EPA model describes the data. The reporting of the mortality data for mesothelioma is generally even less appropriate for risk assessment. Ideally, what is needed is the incidence of mesothelioma subdivided according to exposure level, age at beginning of exposure, and duration of exposure (U.S. EPA 1986, also described in Section 7.3). Such data are almost never available in published studies and crude approximations must be made to account for this lack of information.

It is important to understand the type and magnitude of effect that each of these sources of uncertainties are likely to have on the distribution of potency estimates derived from the set of available studies for lung cancer and mesothelioma, respectively. Some of the above-described limitations likely introduce random errors that simply decrease the overall precision of a potency estimate. However, other types of limitations may cause systematic errors in particular studies, which potentially bias the potency estimate either high or low. Some of the limitations may only affect between-study comparisons and some may introduce a systematic bias between either industry types or fiber types. Examples of some of these types of variation are provided in Section 7.1.

We also note that, although individual estimates of potency factors from individual studies may be highly uncertain, by combining results across multiple studies while properly addressing such uncertainties, it may be possible to draw conclusions with greater precision than reasonable for any individual study. This is the essential advantage of the type of meta analysis discussed in this document (see Chapter 7).

5.2 HUMAN PATHOLOGY STUDIES

Human pathology studies provide a characterization of disease morphology and correlations between causes of death and the types of asbestos fibers retained in the lungs and other bodily tissues. These studies generally involve microscopic examination of tissue samples for indications of morphologic changes characteristic of disease and/or microscopic examination of digested tissue specimens to characterize the mineral fibers extracted from such tissue.

The results of human pathology studies need to be evaluated carefully by addressing effects that are attributable to:

- the way tissue samples are fixed for preservation;
- the way tissue samples are prepared for analysis (e.g., ashing, bleach digestion, digestion in alkali, or some combination);
- the choice of methods employed for characterization of asbestos; and
- the choice of locations within tissues from which samples are collected for analysis.

Because tissue samples obtained from deceased individuals are typically stored for long periods of time before they may be analyzed as part of a human pathology study, such samples are commonly fixed by treatment with chemical preservatives prior to storage. However, Law et al. (1991) studied the effects of two common fixatives (Karnovsky's fixative and formalin fixative) on asbestos fibers and concluded that such fixatives degrade and dissolve asbestos fibers (including both chrysotile and crocidolite) at measurable rates. Therefore, particularly for samples that are stored "wet" (as opposed, for example, to storage in paraffin blocks), the concentrations and character of the tissue burden of asbestos may be altered during storage. Even for studies in which relative (as opposed to absolute) concentrations are being compared, alterations associated with preservation may limit the ability to make such comparisons, particularly among samples stored for widely disparate periods of time or stored using widely disparate procedures.

Fiber concentrations in tissue samples have also been shown to vary as a function of the method employed for preparing such samples for analysis. Historically, samples that are digested in bleach or alkali have tended to exhibit lower recovery of asbestos fibers than samples that are ashed. However, more recent studies suggest that improving technique has narrowed these differences so that this is no longer a major consideration . Thus, when comparing results across studies, due consideration needs to be given for the time frame during which such studies were conducted and the comparability/differences in the techniques employed for tissue sample preparation.

Once prepared, both the character and the concentration of the tissue burden measured in a tissue sample will also depend heavily on the particular analytical method employed to characterize asbestos and differences attributable to such techniques must be reconciled before measurements across samples or conclusions across studies can be reasonably compared. A more detailed

description of the effects attributable to asbestos measurement was presented in the previous section on human epidemiology studies (Section 5.1) and the same issues obtain for human pathology studies. Unless measurements are made using comparable instrumentation with comparable methodology, comparisons across such measurements can be very misleading.

Perhaps the biggest limitation hindering the kinds of evaluation that can be conducted based on human pathology studies is that due to the strong dependence of asbestos concentrations on the specific location within a tissue from which a sample is obtained. Numerous authors have reported that asbestos is non-uniformly distributed in lung parenchyma and other tissues following exposure (see, for example: Bignon et al. 1979; Davis et al. 1986a; Pooley 1982). The incidence of lesions and other pathological effects attributed to asbestos exposure correspondingly exhibit a non-uniform distribution.

For lung tissue samples (which tend to be among the primary interests in human pathology studies) the relationship between sample location and asbestos concentration is particularly important. To sample deep lung tissue reproducibly, it has been shown necessary to select a specific section of lung parenchyma from a defined portion of the bronchio-alveolar tree. Pinkerton et al. (1986) showed that the deposition of asbestos in the lungs is an inverse function both of the path length and the number of bifurcations between the trachea and the site. Thus, analyses of samples from different animals of the same species can only be compared meaningfully if the samples are collected from identical locations in the bronchio-alveolar tree. Similar, nonuniform depositional patterns have also been observed in humans (Raabe 1984). Furthermore, due to the complex branching and folding pattern of the lung, adjacent sections of lung parenchyma frequently represent disparate portions of the bronchio-alveolar tree (Brody et al. 1981; Pinkerton et al. 1986). Consequently, lung burdens derived even from adjacent samples of lung parenchyma can show broadly varying concentrations (differing by orders of magnitude).

Unfortunately, however, tissue samples that are available for analysis in support of a human pathology study are typically “opportunistic” samples, which means that they were selected and stored for an entirely different purpose than the study at hand and, although there may sometimes have been attempts to sample comparable locations across lungs in a general way, this is not adequate for assuring that comparable portions of the respiratory tree are being sampled. It is therefore seldom possible to address the effects of sample location directly. Consequently, comparisons of tissue burden concentrations across samples from different individuals in a human pathology study are at best qualitative and may only be useful when averaged over large numbers of individuals and only when large differences in concentrations (several orders of magnitude) are being distinguished. Moreover, because the parts of a tissue that undergoes morphologic changes induced by asbestos typically corresponds to the parts of a tissue where asbestos burdens are the highest, even comparison of morphologic effects across tissue samples requires proper consideration of the effects of the locations from which such tissue samples were derived.

5.3 ANIMAL STUDIES

In animal studies, members of one of various species (generally rodents) are exposed to measured doses of size-selected mineral fibers and the resultant biological responses are monitored. Animals may be dosed either by inhalation, ingestion, intratracheal installation, implantation, or injection (U.S. EPA 1986). Such studies are conducted for several purposes. As with human pathology studies, animal pathology studies are those in which the transport of asbestos structures is tracked through the various organs and tissues of the animal and the attendant cellular and molecular changes are characterized. In parallel with quantitative epidemiology studies, animal dose/response studies track the incidence of disease among a population that has been exposed in a controlled manner. One of the advantages of animal dose/response studies over epidemiology studies is that exposures are controlled and can be well characterized. The major disadvantage is that there are many uncertainties introduced when extrapolating the results of animal data to predict effects in humans. Therefore, attempts to adapt such things as animal-derived dose-response factors to humans are not generally recommended.

As with human epidemiology and pathology studies, the validity of conclusions drawn from animal studies depends strongly on the techniques and methods used to characterize and quantify asbestos structures either in the delivered dose or in the tissues of the dosed animals (see Section 5.1). The ability to reconcile conclusions derived from many animal studies with the rest of the asbestos literature is limited because SEM was commonly employed to measure asbestos in animal studies, but not other kinds of studies. Even many of those studies in which TEM was employed for asbestos analysis suffer from use of non-standard methods that cannot be easily reconciled with the more traditional TEM methods, particularly because such specialized methods are seldom adequately documented to allow comparison.

As with human pathology studies, the location of a tissue sample excised for analysis is a critical factor that also governs the quality of an animal pathology study (Section 5.2). However, one potential advantage frequently available in animal pathology studies over human studies is the ability to carefully identify and select the precise tissue samples to be analyzed. The extent that a particular animal pathology study exploits this capability can affect the overall utility of the study. Thus, such issues need to be addressed carefully when evaluating and comparing the results across animal pathology studies.

The route of exposure employed in a particular animal study is also important to consider. Each of the routes of exposure commonly employed in these studies (inhalation, ingestion, intratracheal installation, and injection or implantation) delivers different size fractions of asbestos to a target tissue with varying efficiencies. For example, injection or implantation studies deliver 100% of all size categories of structures to the target tissue. However, the efficiency that each size category is delivered by inhalation is a function of the aerodynamic properties of the asbestos structures and the air flow characteristics of the lungs (see, for example, Yu et al. 1991, 1994). Thus, the relationship between dose and exposure depends upon the route of exposure employed. Importantly, because the ultimate goal is to understand the effects that inhaled fibers have on humans, differences between the character of the delivered dose in an animal study and the character that such a dose would have, had it originally been inhaled, typically need to be addressed.

Regarding the measurement of health effects, many of the results in animal studies suffer from a lack of statistical significance because of small numbers of observed tumors. Consequently, trends cannot be established conclusively.

For animal inhalation studies, meaningful comparison of the relative deposition of asbestos dusts across species is not direct. To extrapolate results across species, the detailed differences in the physiology of the respiratory tracts between the species need to be addressed (Section 4.4). However, if measurements are available for both species, differences in physiology are addressed, and the manner in which tissue burdens are analyzed is considered, it may be possible to compare relative tissue doses (mass of asbestos per mass of tissue) across species.

5.4 *IN VITRO* STUDIES

A broad range of *in vitro* studies provide useful insight on the effects of asbestos. These include, for example, studies in cell-free systems (which have been used to evaluate such things as asbestos dissolution rates or the kinetics of free radical formation on the surface of asbestos fibers) and studies of the effects of asbestos on cultures of a broad variety of cell types and tissues.

As with other studies, the potential limitations and sources of uncertainty associated with *in vitro* studies need to be considered when evaluating the validity of study results or comparing such results to those of other studies, particular studies of varying type. Also, as with other studies, among the primary sources of uncertainty that need to be addressed for *in vitro* studies is the manner in which asbestos doses are characterized and quantified (Section 5.1). For *in vitro* studies (as with animal studies dosed by routes other than inhalation), this also extends to the need to consider the relationship between the character of the asbestos dose applied *in vitro* and the character that a similar exposure might possess following inhalation exposure *in vivo* (Section 5.3). This can be particularly problematic for studies in tissue cultures because it is not clear how the application of a suspension of fibers (with known concentration) to a dish containing cultured cells can be related to doses that reach corresponding tissues following administration to whole animals.

In vitro studies, of necessity, represent isolated components of living systems observed under conditions that may vary radically from those under which such components operate *in vivo*. Consequently, the behavior of such components may also vary radically from the behavior of the same components *in vivo*. Therefore, additional study-specific considerations (concerning the design of a study and the conditions under which a study is conducted) also need to be addressed before evaluating the validity or relevance of the results from an *in vitro* study to what might otherwise be observed in a whole animals. Examples of such considerations include:

For cell-free systems

- whether conditions under which the study is conducted are sufficiently similar to conditions *in vivo* to expect that the observed effect is likely to occur *in vivo*; and

- if the observed variables describing the nature or magnitude of the effect are also likely to reflect what may occur *in vivo*;

For tissue cultures

- whether conditions under which the study is conducted are sufficiently similar to conditions *in vivo* to expect that the observed effect is likely to occur *in vivo*;

- whether responses by specific tissues or cells in culture are likely to behave similarly *in vivo* where their behavior may be suppressed, enhanced, or modified in some other manner due to additional stimuli provided by responses of other tissues and cells that are components of a complete organism but that may be lacking in culture; and

- whether the conditions required to establish and maintain a tissue culture for experimentation sufficiently alters the characteristics and behavior of the cells being studied to minimize the relevance of results from such a study to conditions *in vivo*.

Among the most important examples of the last consideration relates to the general need to create immortalized cells to maintain tissue cultures. Thus, questions must always be raised concerning whether the alterations required to create immortalized cells for culture (from what are normally mortal cells *in vivo*) also alter the nature of the responses being studied.

Note, many studies in the current literature also incorporate combined aspects of several of the four general study types described in this chapter. For these studies, a corresponding combination of the considerations described must therefore be addressed when evaluating such studies and comparing their results with inferences derived from the rest of the literature.