Role of Biopersistence in the Pathogenicity of Man-made Fibers and Methods for Evaluating Biopersistence: A Summary of Two Round-table Discussions

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This paper summarizes two roundtable discussions held at the conclusion of the International Conference on Biopersistence of Respirable Synthetic Fibres and Minerals. The first round table addressed the role of biopersistence in the pathogenicity of fiber-induced disease. The panel included T. W. Hesterberg (Chairman), J.M.G. Davis, K. Donaldson, B. Fubini, N.F. Johnson, G. Oberdoerster, P. Sébastien, and D. Warheit. The second panel addressed the issue of methods for assessing biopersistence. It included R.O. McClellan (Chairman), J. Brain, A. Langer, A. Morgan, C. Morscheidt, H. Muhle, and R. Musselman. The two chairmen acknowledge the excellent contributions of all the members of the panels, whose comments formed the basis of this summary. Nonetheless, the authors assume full responsibility for the written text, recognizing that it was not reviewed by the discussants of the two panels. — Environ Health Perspect 102(Suppl 5):277–283 (1994)

Key words: fibers, lung cancer, mesothelioma, biopersistence, fiber dissolution, risk, hazard

Introduction

This article summarizes briefly available scientific information on the role of biopersistence in the pathogenicity of man-made fibers and methods for evaluating their biopersistence. In addition, at the end of this article, we note some of the gaps in our knowledge and make recommendations for future research.

Fiber biopersistence can be defined as the retention in the lung, over time, of fibers with regard to number, dimension, surface chemistry, chemical composition, surface area, and similar physical characteristics. Changes in any of these parameters may alter fiber toxicity. For this article, the term "lung" is used to encompass the respiratory airways, parenchyma, and the pleura. Earlier articles in this volume document the influence of fiber characteristics on toxicity and carcinogenicity, and cover in detail many of the points made here. A summary of a Workshop on Evaluating the Toxicity and Carcinogenicity of Man-Made Fibers has also been published (*1*).

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Fibers can be eliminated from the lung by bulk clearance, primarily involving macrophage uptake and transport to the mucociliary escalator, and by dissolution. Mechanisms for translocation of fibers to the pleura and the clearance of fibers from pleural spaces are not as well understood. *In vivo* fiber instillation experiments have demonstrated that short segments of man-made vitreous fibers (MMVF) are more readily removed from the lungs by macrophages and mucociliary clearance than longer fibers (*2*). The same phenomenon has been demonstrated for chrysotile asbestos following inhalation (*3*). It has also been shown that fibers can be cleared from terminal airways by epithelial cell uptake (*3*), with subsequent translocation into the interstitium and lymphatic system (*4,5*).

Fiber biopersistence is dependent upon the site and rate of deposition, as well as on rates of translocation, clearance, dissolution, and biomodification of the fiber in the lung. It is quite possible that a change in the rate of one of these processes could affect the rates of other processes. For example, a large increase in the rate of deposition in the alveolar region could potentially overwhelm macrophage clearance mechanisms and increase the rate of translocation to the lung interstitium. A better understanding of the biological fate of a fiber in the lung, which is dependent upon all the various fiber characteristics, is critical to understanding the mechanisms underlying the differences in the toxic potential of man-made fibers of different compositions. Before focusing on biopersistence, it is useful to review the factors determining the deposition and biological fate of fibers in the lung.

Deposition

Fiber inhalation and deposition are the initial events in fiber-induced lung disease. Fiber size and geometry are the determinants of both host entry and intrapulmonic distribution, and the essential determinant of host entry is the aerodynamic diameter of the fiber (*6*). Fibers having aerodynamic diameters greater than 12 μm for humans and 6 μm for rodents are not likely to reach the bronchioles and alveoli (*7*). Fibers with diameters less than or equal to 3 μm are considered respirable, even those with lengths as great as 100 to 200 μm (*8,9*). This is because the role of fiber diameter, rather than length, is dominant in determining aerodynamic diameter.

Fibers deposit mainly by impaction, sedimentation, and interception; very little is known about the role of electrostatic precipitation and diffusion in the deposition of fibers (*10*). Both impaction and sedimentation are governed by the aerodynamic diameter of the fibers. Impaction is favored by high airflow velocities and is the predominant mechanism for fiber deposi-

tion in large airways. In contrast, sedimentation is favored by low flow velocity, long residence time, and small airway size. The role of interception increases with fiber length (*8*).

Some authors have developed correlations for the risk of lung cancer associated with surface area, dimensions, and number of asbestos fibers in the lung, since these are the generally accepted determinants of biopersistence (*11*), but such correlations are not available for man-made fibers, although biopersistence may also be an important toxic determinant for them. While fiber dimension determines the entry and deposition in the lung, biopersistence is critical for the accumulation of fibers and for their continuing bioreactivity.

Translocation and Clearance

After a fiber is deposited in the lung, translocation or movement within the lungs can occur. Fibers deposited in the bronchoalveolar region can be translocated to the ciliated epithelium for ultimate bulk removal, or into epithelial cells lining the alveoli and into the interstitium. Subsequently, fibers may be translocated along lymphatic drainage pathways or remain within the interstitium. If the fibers are short enough, phagocytosis by macrophages may be involved in fiber translocation. Fiber dimensions influence translocation, with fibers found at the pleura being shorter and smaller in diameter than those found in the central regions of the lung (*12*).

Recent evidence suggests that the fate and clearance of inhaled fibers deposited in the distal lung may be influenced by their capacity to migrate from the alveolar to interstitial compartments and that all types of fibers do not translocate at the same rate. In a recent inhalation study with Kevlar aramid fibrils, rats were exposed to fibrous aerosols for 1 week and evaluated by electron and light microscopy at 24 hr postexposure. Most Kevlar fibrils that had deposited in the alveolar regions were observed within alveolar macrophages. Some macrophages that had phagocytized fibrils adhered to Type I epithelial cells (*13*), in contrast to previously observed effects in the lungs of chrysotile asbestos-exposed rats, where chrysotile fibers readily translocated from airspaces through epithelial cells to the interstitium (*3*). This difference may have significant implications for lung clearance responses, as well as for the development of interstitial fibrosis. It seems likely that durable types of fibers that translocate to interstitial compartments will be cleared more slowly and may be more fibrogenic, due in part to the inability of these fibers to reach the alveolar macrophages and the mucociliary escalator. Moreover, fibers that reside for a longer time within interstitial compartments may contribute to pathogenic events underlying the development of interstitial pulmonary fibrosis. In contrast, nondurable fibers that translocate from airspaces to the interstitium may be cleared from the lung by dissolution processes in interstitial or lymphatic compartments or within the cytoplasm of interstitial macrophages.

Dissolution

Evidence of *in vivo* dissolution of MMVF in the lungs has been extensively reviewed (*14,15*). It has been demonstrated that the dissolution of inhaled MMVF is dependent on fiber length, which may be due to differences in extracellular and intracellular pH and to the chemical composition of the fibers (*16,17*). The relationship between *in vivo* solubility and the chemical composition of inhaled fibers has been demonstrated for a variety of MMVF, such as refractory ceramic fibers (RCF), glass fiber, rockwool, mineral wool, slagwool, chrysotile asbestos, and amphibole asbestos (*15–21*). Their relative solubilities, set in decreasing order, are glass fiber, slagwool > rockwool > RCF > chrysotile asbestos > amphibole asbestos. Their toxicological potentials appear to increase in the same order.

Chronic Inhalation Studies

Relatively few long-term inhalation studies with either naturally occurring or man-made fibers have been conducted (*1*). Fortunately, new technology for conducting such exposures, as described elsewhere (*22–24*), provides the opportunity to conduct inhalation studies that meet standards previously not attainable. In one chronic inhalation study, the biological effects on rats of long-term exposure to glass fibers (FG) (*23*) were compared with the effects of exposure to chrysotile asbestos and to RCF (*25*). The glass fibers, which were of similar composition to common building insulation wools, produced no lung fibrosis in the exposed rats even after 24 months at a concentration of 30 mg/m^3, neither were there any mesotheliomas nor any significant increase in lung tumors. In contrast, exposure to chrysotile and, to a lesser extent, to RCF, resulted in lung fibrosis, mesotheliomas, and a significant increase in lung tumors.

These findings are significant because observed lung burdens and dimensions of FG were comparable to those of RCF 1-exposed animals, yet FG did not induce lung fibrosis or tumors. This suggests that internal dose and dimension are not sufficient to explain the toxic potential of two chemically different types of fibers. Chemical composition and surface physicochemical properties also may be important determinants of fiber toxicity for similarly sized fibers. The importance of other fiber characteristics, such as chemical composition, surface charge, and biological persistence as determinants of fiber toxicity is now well recognized (*1,15,20,22*).

Methodological Issues

It is important to consider methodological issues related to the administration of the test material and to the recovery of fibers from lung tissue to study the lung burden, biopersistence, and toxicity of fibers. Although intratracheal instillation permits precise dosage and is economical, the suitability of this model for fiber toxicity evaluation is still debatable. Distribution and deposition patterns within the lung airways after intratracheal instillation may not be the same as after inhalation. For example, intratracheal instillation of suspended fibers can result in granulomas containing fibers in the upper airways (bolus effect), although this was overcome by using fiber suspensions at low concentrations (*18*). Validation by a comparison of instillation and inhalation studies is certainly necessary, and intracavitary administration of fibers should also be included in the comparisons.

The low durability of MMVF in comparison to asbestos poses special problems in the storage of exposed lung tissue and subsequent fiber recovery. Storage in a fixative of tissue containing MMVF can alter the chemical composition of the fiber (*26*), as can some wet tissue digestion techniques, which can also alter physical dimensions of fibers (*27*). Techniques need to be developed that are appropriate for each fiber composition, and wet digestion techniques require special caution in the selection of reagents. We believe that the preferred technique for recovering MMVF from lung tissue should start with immediate freezing of the tissue for storage. The recovery process would then involve thawing the tissue, followed by rapid dehydration with acetone, low temperature ashing, and then rapid dispersion in distilled water, followed by immediate collection onto filters for microscopic analysis. In any

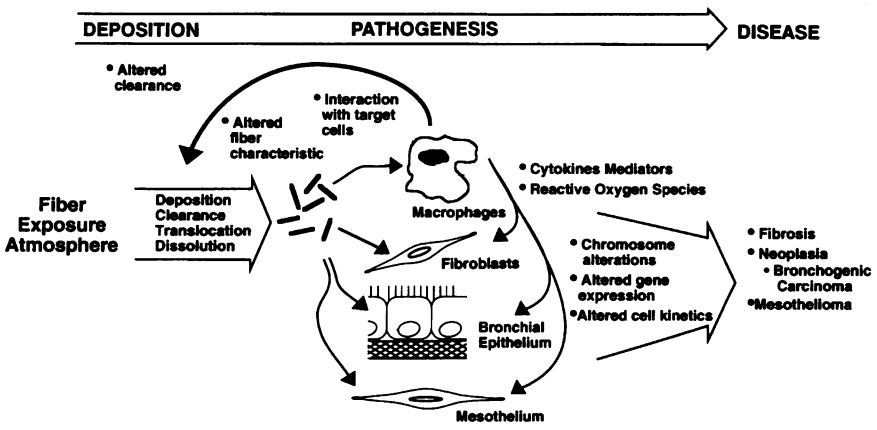

Figure 1. Schematic representation of mechanisms of fiber-induced disease.

case, validation would be essential for any proposed technique.

Summary of Knowledge

We have attempted here to summarize the current state of knowledge of the biopersistence of man-made fibers in the respiratory tract and the consequent biological response. This body of information rests not just on the small number of investigations conducted with relatively few man-made fibers, but also on the more extensive knowledge obtained from studies of asbestos fibers. A general representation of the mechanisms by which fibers, such as certain types of asbestos, induce pulmonary disease is shown in Figure 1. Although man-made fibers have been less well studied, it is presumed that those that cause disease operate by similar mechanisms. Not all man-made fibers are capable of producing disease, neither are all forms of asbestos equally toxic in respect to the different pathological end points (*11,28–30*).

Strategy for Evaluating Biopersistence and Its Significance

Our interest in biopersistence is twofold. First, an understanding of the biopersistence of man-made fibers is essential to an understanding of the mechanisms by which inhaled fibers may or may not cause cancer or other diseases of the respiratory tract. Second, given that biopersistence has a significant role in the pathogenesis of fiber-induced diseases, including cancer, evaluation of biopersistence may provide useful surrogate marker(s) for evaluating the disease-causing potential of any man-made fibers being considered for introduction to commerce. Obviously, the ideal would be a complete understanding of how inhaled fibers cause disease and the identification of all necessary and sufficient steps, including those relating to biopersistence. With that level of knowledge, it would then be possible to develop procedures to evaluate the potential of any fiber to initiate any of those steps.

Unfortunately, our present knowledge of the mechanisms by which fibers cause disease is incomplete; moreover, it has emerged primarily from studies of asbestos-exposed people and laboratory studies with various kinds of asbestos and man-made fibers (Figure 1). Our confidence in this schematic model as a descriptor for asbestos is greatly strengthened by actual observations of human disease as the endpoints of concern—pleural and lung fibrosis, bronchogenic carcinomas and mesotheliomas. Similar diseases have been observed in asbestos-exposed laboratory animals, which, in a sense, validates the laboratory animal model. A paradigm for extending our knowledge to man-made fibers is shown in Figure 2. The aim is to study man-made fibers and apply the knowledge gained early enough to establish appropriate control procedures that would prevent fiber-induced disease in people and so, fortunately, limit the potential for observing the effects on humans.

The development of more structured exposure-dose-response models is most necessary (Figure 3), as well as validated experimental systems in which the end diseases of concern observed in laboratory animals could be confidently extrapolated to humans.

For man-made fibers, the nature of the data base associating exposure and disease is inconclusive, so that it is not possible to validate animal experiments or other laboratory studies by comparing results with demonstrated effects in exposed humans. Fortunately, the various methods of evaluation have been indirectly validated, by reference to the data bases available on asbestos. If disease were observed in laboratory animals exposed to man-made fibers, this would need to be viewed as a positive indicator of the potential health hazard for man. Conversely, while the absence of disease in well-conducted studies with laboratory animals exposed to man-made fibers cannot be used as evidence for a lack of hazard for man if similarly exposed, it certainly supports of this conclusion.

This viewpoint is summarized schematically in Figures 4 and 5. In Figure 4, each step in the multistep process leading to an adverse disease outcome may serve as the basis for an evaluation procedure and a

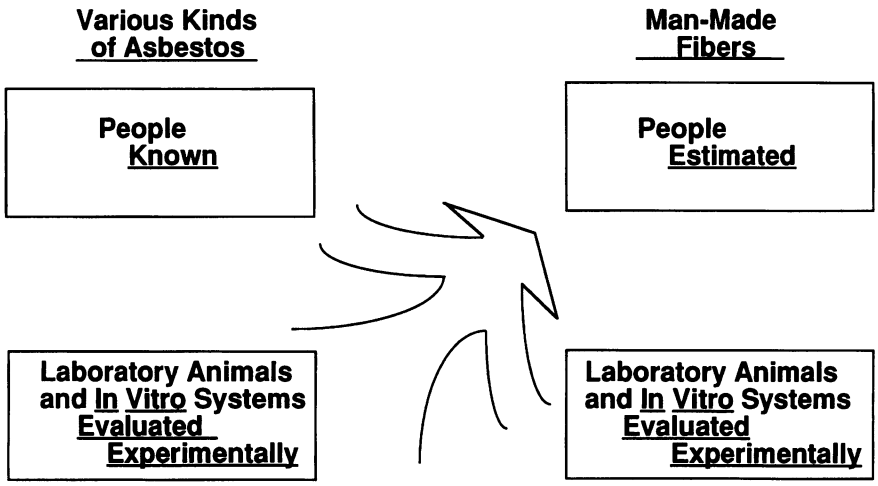

Figure 2. Interrelationship among information acquired from epidemiological studies of asbestos-exposed people, laboratory studies for asbestos, and man-made fibers and estimated human risk from man-made fibers.

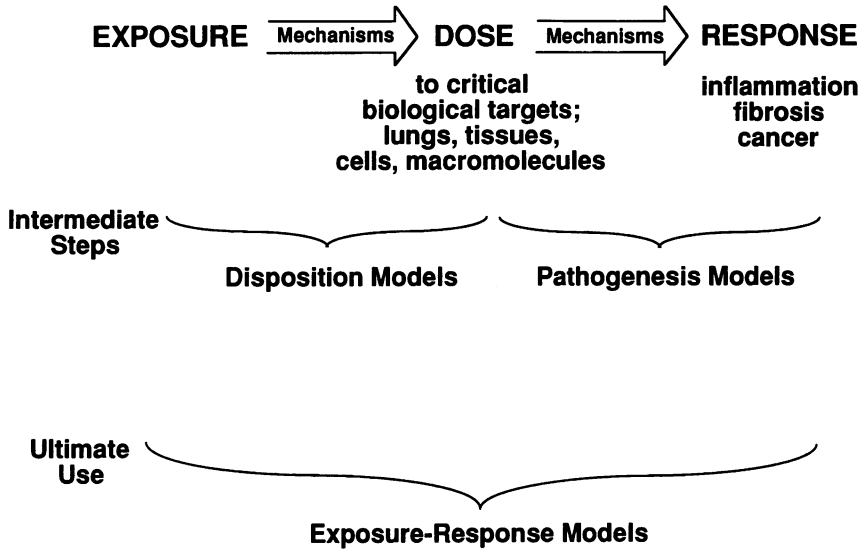

Figure 3. Role of mechanistic data as a base for developing exposure-response models.

number of these steps relate to biopersistence. For example, can the fiber be inhaled and deposited? Do the fibers dissolve rapidly? Do certain chemical constituents selectively leave the fibers? Do the fibers change in physical dimensions? Are the fibers cleared by physical transport processes from the lungs? Do the fibers translocate to the pleural surfaces? Do the fibers enter target cells? Are the fibers cytotoxic to target cells? Do the fibers cause release of cytokines or mediators? These questions can be studied in detail in experimental systems with the expectation that the answers can ultimately be extrapolated to human populations (Figure 5).

This long list of questions is by no means complete, which is, in itself, a reflection of our level of knowledge of fiber-induced disease. Moreover, many of the processes involved would not yield a yes or no answer but rather fall within a continuum, for example between slow and rapid dissolution. Therefore it is unlikely that any single evaluation procedure related to biopersistence will provide a yes or no answer about the potential of a test fiber to cause cancer. It is important to consider the outcome of any evaluation procedure in a quantitative, probabilistic manner, including taking account of the dose of test material administered. Certain carcinogenicity studies can be considered to illustrate this point.

It is generally agreed that the greater the durability of a fiber type, the greater is its likelihood of causing disease. Conversely, the lower the durability, the lower is the disease-causing potential, so that fibers that dissolve relatively rapidly should have lower potential for causing disease.

In a test of an experimental fiber of moderately high solubility by intracavitary instillation in rats, tumors were observed only at very high dose level (*30*). Interpretation of this result must take into account the mode of administration as well as the dose administered, but would suggest that even soluble fibers might be carcinogenic if the fiber dose were high enough. Special care is required in making such an interpretation based on a single, and perhaps overly simplified, procedure.

These considerations also point to the need to shift our orientation from asking whether the material is carcinogenic to asking what is the potential cancer risk at anticipated levels of human exposure. This kind of orientation is inherent in the use of exposure–response models (Figure 3). It also requires careful consideration of likely human exposure circumstances throughout the product life cycle (Figure 6). The fiber level and characteristics used in experimental studies should be linked to the fiber level and characteristics at each stage of potential human exposure. This should be borne in mind, for example, where fiber samples are subjected to size separation prior to experimental use, to obtain a higher proportion of respirable fibers than would probably be encountered in the occupational or environmental setting.

	Exposure → Disposition → Pathogenesis → Disease								
	Steps in the carcinogenic process								
	Possible Exposure	1	2	3	4	5	n	Laboratory Animals	Humans
Asbestos									
People	+	+	+	+	+	+			+
Laboratory Animals	+	+	+	+	+	+		+	
Test Fiber in laboratory animals									
A	+	+	+	+	+	+		+	(a)
B	+	+	+/−	−	−	−		−	(b)

+/−, the presence (+) or absence (−) of a given step or the end disease

(a), presumptive positive evidence for human carcinogenicity

(b), presumptive negative evidence for human carcinogenicity

Figure 4. Validation of fiber evaluation procedures for estimating human health risks.

Tiered Approach

To obtain information on new fibers as quickly as possible and to keep testing costs moderate, a tiered approach to evaluating new fibers was recommended by participants in a previous workshop (*1*). This approach (Figure 7) has the advantage that the first steps can be completed at low cost and within months, in contrast to the conduct of long-term inhalation studies that would be expensive and require a minimum of 3 to 4 years to complete. With the

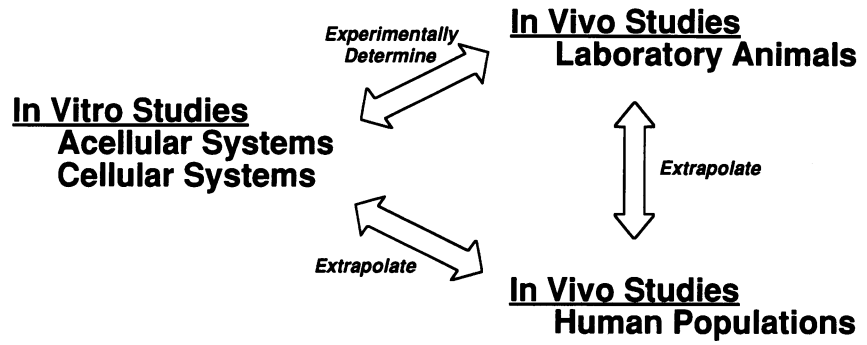

Figure 5. Critical linkages in understanding potential toxicity and carcinogenicity of man-made fibers.

tiered approach, a number of test fibers could be evaluated with the first few steps, which might then identify some fibers that have unfavorable characteristics and responses that suggest such a high potential for toxicity or carcinogenicity that it would be inappropriate to consider further commercial development, especially if the fiber product cycle is likely to involve significant human exposure. Conversely, it may also ultimately be possible to identify early in the screening tiers those fibers that have favorable characteristics and negative responses indicating that they would present a minimal potential hazard, thus obviating the need for further evaluation. Unfortunately, the level of confidence in the screening procedures is, at present, not sufficient to permit this course of action, and it is necessary to evaluate fibers in long-term inhalation studies if they have high potential for commercial use and if the product life cycle indicates the likelihood of human exposure.

There has been extensive and vigorous discussion at this conference on the role of long-term inhalation studies versus intracavitary injection studies for hazard identification or risk characterization. Most scientists view inhalation as the preferred route of exposure, since it simulates human exposures and results in fibers reaching the target tissues at an appropriate rate relative to potential human exposures. Others (*31*) advocate use of intracavitary injection to assure that a maximum number of fibers reaches one of the target tissues, the pleura; they consider that the inhalation route is not sufficiently sensitive for detection of the neoplasms of concern, which have a long latent period. Conversely, the criticism is made that the intracavitary studies yield false-positive findings because high levels of exposure result from delivery of a bolus or a series of boli of fibers to the cavity. The boli overwhelm normal defense mechanisms, such as macrophage-mediated clearance.

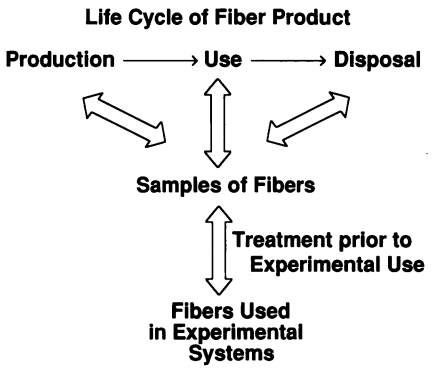

Figure 6. Relating characteristics of fibers studied back to characteristics of fibers during product life cycle.

Future Research Needs

Generic Research

The most critical research needs are for the acquisition of information that will provide mechanistic linkages between exposure and dose, and between dose and response (Figure 3). To be most useful, this should include information acquired from studies conducted at multiple exposure levels and durations, so that convincing evidence may be obtained as to the likely mechanisms of action at relevant potential human exposure levels.

There is, first of all, need for improved techniques for characterizing fiber exposures, including both potential human exposure circumstances within a product life cycle and experimental exposures (Figure 6). It will be especially useful to develop techniques to characterize fibers not only by their dimensions, as has been traditional, but also by surface area and chemistry, which may have special toxicological relevance.

Second, there is a critical need for improved information on the disposition of inhaled fibers both in laboratory animals and humans. Much of our current understanding has been derived indirectly from a much more abundant knowledge base relating to inhaled particles. The techniques are now at hand to develop a similar body of experimental data on the influence of fiber diameter and length on initial deposition and clearance of fibers. Such data are needed on the more frequently used laboratory animal species and, ideally, on people, to enhance our ability to make extrapolations from laboratory animals (Figure 2). The emerging technology should make it possible to obtain much more detailed knowledge on the clearance of fibers of varied size and chemical composition, and to develop models of the regional fate of fibers in the lung and the relative role of physical removal and of dissolution. Once the models have been validated, it will be possible to consider "dose terms" that move beyond the total lung to specific regional tissues and cells.

Third, we need research to link our emerging knowledge of fiber dosimetry with the various steps in the pathogenesis of fiber-induced disease, and to develop a better understanding of the relationship of results obtained *in vitro* to *in vivo* observa-

Tier	Approach	
I	Product life cycle evaluation	
	Physical characterization	
	length & diameter	
	surface area (both internal and external)	
	Chemical composition	
II	*In vitro* acellular systems	
	dissolution	
	durability	
	In vitro cellular studies	
	cell culture	
	cytotoxicity	
	phagocytosis	
	chromosomal damage	
	mediator release	
III	Short-term inhalation studies	
	fiber disposition	
	deposition (fractional/regional)	
	clearance	
	translocation	
	toxicity	
	bronchoalveolar lavage	
	cell/biochemical indicators	
	pathology	
IV	Long-term inhalation studies	
	fiber disposition	
	deposition	recovered and
	clearance	visualized
	translocation	fibers
	toxicity	
	pathology	
	carcinogenicity	
	pathology	
	molecular evaluation	

Figure 7. Tiered approach to characterizing toxicity and carcinogenicity of new fibers.

tions, extending over the life-span of the laboratory animal species up to the appearance of neoplastic diseases. It would be especially useful if comparative studies could be conducted with carcinogenic and noncarcinogenic materials. This approach (Figure 4) would help to identify the critical steps in the process of fiber carcinogenesis. Particular attention still needs to be given to understanding the relevance of observations made with the different models of fiber administration—intracavitary or intratracheal or by inhalation. The continuing debate over the assertions that inhalation studies yield false negatives and that intracavitary studies yield false positives must be resolved.

Finally, we need continued development of improved models (Figure 3) to describe in quantitative terms the relationships between exposure and dose and response that are based on a sound understanding of the biology and pathobiology of the systems being described. Models have great potential not only for integrating and synthesizing what is known but also for identifying deficiencies in our knowledge that may be removed through targeted experimentation.

Research Related to Biopersistence

A number of issues related more specifically to biopersistence have already been discussed. The concept of biopersistence—the retention of fibers in the lung over time—is evidently an important determinant of the toxicity and carcinogenicity of fibers; yet our current knowledge of its significance and the lack of standardized approaches for assessing it are major impediments to using information on biopersistence to make decisions on the potential hazard of a given fiber type. Research on factors such as fiber number, dimension, surface chemistry, chemical composition, surface area, and other parameters that influence biopersistence will change this situation, taking advantage of life-span *in vivo* studies with different kinds of man-made fibers that have yielded both positive and negative carcinogenic results (Figure 4, test fibers A and B). Studying the various steps in the underlying biological processes and how they relate to the physical and chemical characteristics of the fibers will help us to understand which of these characteristics are essential to the carcinogenesis process.

The prospects for progress will be further enhanced as more man-made fibers are evaluated in life-span *in vivo* bioassays, of comparable quality to those recently conducted, and in which it will be especially important to understand as completely as possible the full exposure–dose–response paradigm. In addition to studies with man-made fibers, animal life-span–bioassay studies with asbestos fibers of different types using contemporary inhalation exposure technology and methods will be of unique value. They would provide a linkage to the data base acquired from epidemiological studies of asbestos-exposed populations (Figures 2,4). With these approaches, it should be possible in the not-too-distant future to identify characteristics of biopersistence linked to the carcinogenicity of fibers that would be evaluated in short-to-intermediate-term studies and so could serve as indicators of the carcinogenic potential of newly developed fibers. The degree of confidence in these indicators will depend on the extent to which the assay systems have been validated by comparing fibers that produce cancer with those that do not.

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