
Use of Mechanistic Data in Assessing Human Risks from Exposure to Particles

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The ultimate goal of toxicologic investigations of both natural and man-made fibrous and nonfibrous particles is to provide essential input for the assessment of potential human risks from exposure to these materials. The development of risk assessment procedures for airborne particles has evolved over the years. The earliest assessments for naturally occurring materials used direct human observations and incorporated safety factors to arrive at allowable human exposures. More recently, there has been a need to assess the potential risk associated with production and use of certain man-made materials for which human data are not available or are inadequate. For these materials, it has been necessary to assess human risks using data obtained from studies conducted in laboratory animals and with cells or tissues. During the last several decades, it has been suggested that data on the mechanisms by which particles cause disease could be used to reduce the uncertainty in estimates of human risks of particle exposures. This article provides comments on the use of mechanistic data in the risk assessment process and suggestions for increasing the successful development and use of mechanistic data in risk assessments conducted in the future. — *Environ Health Perspect* 105(Suppl 5):1363–1372 (1997)

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Introduction

Particulate matter has long been identified as noteworthy when considering air quality and its impact on health. Attention initially focused on occupational exposure in dusty trades such as mining, quarrying, and stone masonry. Early in this century, the observation of pulmonary disease associated with asbestos exposure raised concern about fibers as a special kind of particulate material. In this article, the term particulate matter (PM) is used for both nonfibrous and fibrous particulates unless specifically noted otherwise. PM was considered to be

involved in debilitating pulmonary diseases that were manifested by irritation, inflammation, fibrosis, and functional impairment. Perhaps the best example is silicosis, the specific form of pneumoconiosis attributed to exposure to crystalline silica. Other pneumoconioses were attributed to specific dusts and named accordingly. Some dusts, initially termed inert or nuisance dusts and now called particulates not otherwise classified or regulated, produced effects that were reversible if exposures were controlled to modest levels.

Coincident with the steady rise in lung cancer rates that began in the 1930s in men, and later in women, was the awareness that lung cancer might be associated with PM exposures. Cigarette smoking is now known to be the primary risk factor responsible for the increase in lung cancer rates for men that continued until 1990 before slowly beginning to subside (and are continuing to rise for women). Several other specific occupations or agents are also now known risk factors for lung cancer, including asbestos, which is a risk factor for both lung cancer and mesotheliomas.

Concern for lung disease associated with occupational exposures led to early voluntary efforts on the part of industry and local and state authorities to control exposures to PM. These efforts were followed by voluntary national efforts and, ultimately, federal statutes directed toward controlling occupational exposures. The passage of federal statutes for occupational exposure was paralleled by statutes directed toward limiting environmental exposures.

In this article, I briefly review the development of approaches to characterizing human health risks of exposure to PM as a basis for setting exposure limits. I then comment on how mechanistic information is being used in the risk assessment process and offer some suggestions on how the development and utilization of mechanistic information on PM might be improved.

An underlying premise of this article is that PM exposures should be held to the lowest practicable level and that determination of this level can be greatly facilitated by mechanistically understanding how and at what levels of exposure various chemical forms and sizes of PM affect humans. Establishment of standards for occupational and environmental exposures involves use of all of the available scientific data as well as considerable judgment. Ultimately, in most situations, there is consideration of the anticipated health benefits of a standard and the costs of implementing it. My call for the acquisition and use of scientific data should not be viewed as a call for delaying risk management decisions in the face of convincing evidence of human hazard. It is, however, a call for acquiring scientific information that will reduce the uncertainty in assessing human risks and thus increase the certainty that risk management decisions will produce results in a cost-effective manner.

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Abbreviations used: ACGIH, American Conference of Governmental Industrial Hygienists; CAA, Clean Air Act; CASAC, Clean Air Scientific Advisory Committee; HAP, hazardous air pollutant(s); IARC, International Agency for Research on Cancer; NAAQS, National Ambient Air Quality Standard(s); NRC, National Research Council; NTP, U.S. National Toxicology Program; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit(s); PM, particulate matter; PM_{2.5}, particulate matter = 2.5 μm in aerodynamic diameter; PM₁₀, particulate matter ≤ 10 μm in aerodynamic diameter; RfC, reference concentration; TLV, threshold limit value(s); TSCA, Toxic Substances Control Act; TSP, total suspended particulate(s); TWA, time-weighted average(s); U.S. EPA, U.S. Environmental Protection Agency.

Historic Development of Exposure Guidance and Standards

Early occupational standards for PM were of a voluntary nature and were developed by industry and local and state governments. Out of these efforts came the development of the American Conference of Governmental Industrial Hygienists (ACGIH). As early as 1946, the ACGIH issued guidelines for airborne concentrations of various substances, including PM.

The earliest values were presented as maximum allowable concentrations—time-weighted average(s) (TWA), which soon became threshold limit value(s) (TLV)—TWA. TLV are regularly updated, and the current values are given in the ACGIH document *Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices* (1). Supporting documentation is also available (2) that includes brief summaries of the basis for each of the TLV. In the introduction to these documents, TLV are defined: “TLV refer to airborne concentrations of substances and represent conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse health effects.” The TLV documents include a number of specific chemicals found in the air as PM. In addition, a listing is given for particulates not otherwise classified, which was formerly identified as nuisance dust.

The ACGIH is a professional society and not a government agency. Nonetheless, its guidance is widely viewed as authoritative and, as a result, has had substantial impact on workplace practices in the United States and abroad.

Heightened concern for occupational and environmental health issues beginning in the mid-1960s gave impetus to the passage of several major pieces of legislation that established guidance for PM in occupational and environmental settings. Legislation included the Clean Air Act (CAA), the Occupational Safety and Health Act, the Mine Safety Act, the Toxic Substances Control Act (TSCA), Superfund, and the Resources Conservation and Control Act. A review of the details of these various statutes is beyond the scope of this article. Note that major authority related to occupational exposures is vested in the Department of Labor: the Mine Safety and Health Administration is responsible for miners, and the Occupational Safety and Health Administration (OSHA) has more general

responsibility. Major authority related to environmental exposure to airborne materials is vested with the U.S. Environmental Protection Agency (U.S. EPA) under the CAA. The U.S. EPA also influences workplace practices under other statutes, such as TSCA.

In regulating workplace exposures, OSHA utilizes permissible exposure limit(s) (PEL) that are very similar to TLV. Indeed, to jump-start the development of PEL, OSHA originally adopted as PEL the TLV that had been established by the ACGIH. The U.S. EPA broadly regulates air pollutants within two separate sections of the CAA. One of these sections covers the criteria pollutants for which EPA must set National Ambient Air Quality Standard(s) (NAAQS). One of these criteria pollutants is PM. A separate section of the act concerns regulation of hazardous air pollutant(s) (HAP).

Beyond the above-mentioned agencies, the Secretary, Department of Health and Human Services, is responsible for preparing the *Biennial Report on Carcinogens*, (3) which lists agents classified as human carcinogens or reasonably anticipated to be human carcinogens. This task is conducted by the U.S. National Toxicology Program (NTP), which does not have regulatory authority. However, the NTP report is frequently used by other agencies as a basis for regulatory decisions on the listed chemicals. On the international front, the International Agency for Research on Cancer (IARC) periodically prepares monographs that report on the classification of agents or exposure situations as to their carcinogenic potential. The IARC reports are frequently used by national bodies as a basis for regulatory actions.

Risk Assessment Approaches

Three basic approaches are used by the above organizations in developing standards concerning human health risks from exposure to chemicals, including PM. For ease of communication, these approaches are referred to hereafter as *a*) threshold, *b*) cancer classification, and *c*) quantitative estimation of risk approaches. They evolved historically in the order listed.

Threshold Approach

The earliest efforts to assess human health risks of PM used a threshold model. Examples are the TLV set by the ACGIH for crystalline silica and other forms of silica, asbestos, and particulates not otherwise

classified. The TLV are based on information from industrial experience, experimental human and animal studies, or a combination of all three when possible. Obviously, human data are used to the extent that they are available. For materials widely used in commerce, substantial human data may exist. For newly developed materials, human data may be inadequate or nonexistent, requiring heavy reliance on laboratory animal data.

Safety factors or uncertainty factors are used to make extrapolations from laboratory animal data to humans. Typically, 10-fold factors are used to account for interindividual variability in susceptibility, extrapolation from animals to humans, or extrapolation from subchronic to chronic exposures. For example, in a subchronic study conducted in rats, extrapolation of a no observed effect level from rats to humans would involve an overall safety or uncertainty factor of 1000.

The U.S. EPA approach for developing inhalation reference concentrations (RfC) has many similarities to that used to develop the TLV. Jarabek (4) recently reviewed this methodology in detail and defined an RfC as “an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to a human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious noncancer health effects during a lifetime.” The RfC methodology is being used by the U.S. EPA to assess noncancer risks of HAP and other materials for which the agency has statutory responsibility.

The U.S. EPA also uses a threshold approach, customized to the specific pollutant under consideration, in setting the NAAQS for criteria pollutants. In setting the NAAQS, the agency is charged with protecting the general public, including sensitive subpopulations, from adverse health effects, with an ample margin of safety. The ample margin of safety is included to account for uncertainties in current knowledge. Each NAAQS involves four key decisions: selection of an indicator (i.e., what is to be measured), a concentration level, an averaging time, and a statistical form (e.g., one exceedance [instance where standard levels are exceeded] per year).

The U.S. EPA has prepared an updated criteria document and staff position paper on PM (5,6), and has proposed the addition of PM 2.5 μm in aerodynamic diameter (PM_{2.5}) standards to complement the

present $PM \leq 10 \mu m$ in aerodynamic diameter (PM_{10}) standards (7). The proposed change in the PM standards must be considered in a historic context. The original NAAQS for PM promulgated in 1971 was based on total suspended particulate(s) (TSP). This represented all the PM sampled with a high volume sampler [Figure 1; (8)]. This includes particles as large as 30 to 40 μm in aerodynamic diameter. During the late 1970s and early 1980s researchers recognized that the TSP indicator was not particularly health relevant, since many of the particles included in a TSP sample were sufficiently large that they had a low probability of being inhaled and deposited in the respiratory tract.

In 1979 the U.S. EPA announced that it was beginning a review of the PM NAAQS. Over the next 7 years (1979–1986), there was extensive interaction among the scientific community, the U.S. EPA staff, and the agency's Clean Air Scientific Advisory Committee (CASAC) as the PM NAAQS review proceeded. The collaboration included preparation of a criteria document, staff paper, and supplements to both documents. The criteria document is an encyclopedic compilation of all available peer-reviewed literature on the pollutant (PM in this case). The staff position paper is a critical analysis of the literature that is most relevant for identifying the indicator (TSP, PM_{10} , or $PM_{2.5}$), the averaging time (annual, 24 hr), the level of concentration, and the statistical form (arithmetic average,

number of exceedances of the standard, etc.). The CASAC is an independent committee of scientists charged under the CAA with advising the U.S. EPA Administrator on the science used to establish the NAAQS. In 1987 the U.S. EPA changed from the TSP indicator to a more health-relevant PM_{10} indicator (9). Two standards were set: a) an annual standard of $50 \mu g/m^3$ expected arithmetic mean, averaged over 3 years, and b) a 24-hr average standard of $150 \mu g/m^3$, with no more than one expected exceedance per year.

In response to a court-ordered schedule, in 1995 and 1996 the U.S. EPA completed an updated criteria document and related staff paper on PM (5,6). As required by the CAA, the U.S. EPA's CASAC reviewed both documents (10,11).

An overview of the issues involved in revising the PM standards has recently been published (12). Following the PM_{10} standard set in 1987, a number of new epidemiologic studies were published showing an association between TSP or PM_{10} levels and various indices of morbidity and mortality. Exposure data for the TSP and PM_{10} indicators were available because of regulatory requirements for TSP measurement (up until 1987) and for PM_{10} measurement (beginning in 1987). Meager data are available using a $PM_{2.5}$ indicator. Interpretation of the epidemiologic studies is complicated by the difficulty of separating effects of TSP, PM_{10} , or $PM_{2.5}$ from the effects of other air pollutants

such as ozone and carbon monoxide and confounders such as weather and cigarette smoking. In the establishment of NAAQS, data from controlled exposure studies with human subjects and experimental studies with laboratory animals, tissues, and cells are typically used to complement data from epidemiological studies. Unfortunately, in the case of PM_{10} and $PM_{2.5}$, the complementary data are very limited.

Based on the available PM data, the U.S. EPA staff paper recommended the establishment of $PM_{2.5}$ standards to complement the PM_{10} standards (6). Specifically, the recommendation was for a 24-hr average $PM_{2.5}$ standard in the range of 18 to $65 \mu g/m^3$ and an annual $PM_{2.5}$ standard in the range of 12.5 to $20 \mu g/m^3$. The staff also recommended continued use of a PM_{10} indicator: $150 \mu g/m^3$ for a 24-hr average and 40 to $50 \mu g/m^3$ for an annual standard. Nineteen of 21 CASAC members endorsed the establishment of a $PM_{2.5}$ indicator. Two individuals were opposed to the use of a $PM_{2.5}$ indicator, and were joined by two others opposed to the setting of a $PM_{2.5}$ 24-hr standard and six individuals opposed to a $PM_{2.5}$ annual standard. Individual CASAC members expressed a wide range of opinions as to the appropriate level for various standards [Table 1 (11,13)].

The diversity of opinions expressed by CASAC members reflects the high degree of uncertainty in our current knowledge of PM effects, and especially those that can be quantitatively linked to $PM_{2.5}$. These uncertainties include a) the influence of confounding variables such as other pollutants, b) measurement errors, c) exposure misclassification, d) the lack of understanding of toxicologic mechanisms to explain the effects, e) the use of different models in various studies, and f) the shape of the exposure–response function for typical exposures currently experienced in the United States.

In late 1996 the U.S. EPA proposed revisions to the PM NAAQS (14). It was proposed that the PM_{10} standards would be retained; however, the one expected exceedance form of the $150 \mu g/m^3$ 24-hr standard would be changed to a 98th percentile form averaged over 3 years. Most significantly, the agency proposed new $PM_{2.5}$ standards set at $15 \mu g/m^3$ annual mean, and $50 \mu g/m^3$ 24-hr average. The annual standard would be based on the 3-year average of the annual arithmetic mean $PM_{2.5}$ concentrations, spatially averaged across an area. The 24-hr average would be based on a 3-year average of the 98th

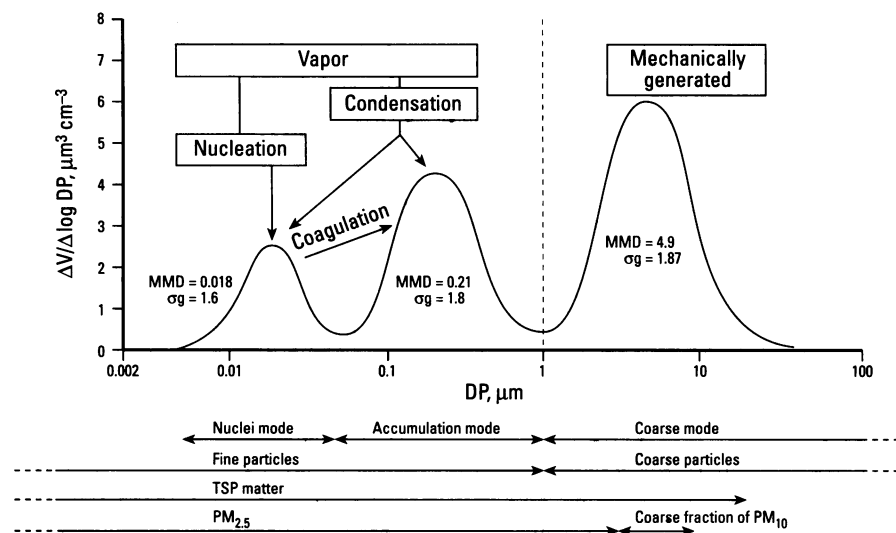


Figure 1. Schematic of the relationship between various parameters used to describe the size distribution of airborne particles. Adapted from Wilson and Suh (8). Abbreviations: σ_g , geometric deviation; DP, diameter of particle; MMD, mass median diameter; V, volume.

Table 1. IARC carcinogen evaluation scheme.^a

| Category | Human evidence | Experimental animal evidence |
|---------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Group 1. The agent (mixture) is carcinogenic to humans. The exposure circumstance entails exposures that are carcinogenic to humans. | (a) Sufficient (b) Less than sufficient | No animal evidence required Sufficient evidence and strong evidence in exposed humans that the agent (mixture) acts through a relevant mechanism of carcinogenicity |
| Group 2A. The agent (mixture) is probably carcinogenic to humans. The exposure circumstance entails exposures that are probably carcinogenic to humans. | (a) Limited (b) Limited (c) Inadequate | None Sufficient Sufficient and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans |
| Group 2B. The agent (mixture) is possibly carcinogenic to humans. The exposure circumstance entails exposures that are possibly carcinogenic to humans. | (a) Limited (b) Inadequate (c) Inadequate | Less than sufficient Sufficient Limited, with supporting evidence from other relevant data |
| Group 3. The agent (mixture or exposure circumstance) is not classifiable as to its carcinogenicity. | (a) Inadequate (b) Inadequate | Inadequate or limited Sufficient and strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans |
| Group 4. The agent (mixture) is probably not carcinogenic to humans. | (a) Lack of carcinogenicity (b) Inadequate | Lack of carcinogenicity Lack of carcinogenicity consistently and strongly supported by a broad range of other relevant data |

^aAdapted from IARC (13).

percentile of 24-hr PM_{2.5} concentrations at each monitor within an area.

This U.S. EPA proposal generated much discussion. One point of controversy is apparent from consideration of the exposure–response functions shown schematically in Figure 2. In the exposure range of interest, it is difficult to establish whether there is a threshold exposure level that must be exceeded before effects are observed. In addition, the lack of information on the mechanisms by which PM is causing effects at low levels of exposure does not provide a mechanistic basis for selecting one exposure–response function over another.

The choice of a linear model of exposure and response including PM_{2.5} concentrations down to 5 µg/m³, compared to the use of a threshold concentration of 18 µg/m³, is illustrated in Figure 3 (14). The assumption that linearity extends down to very low PM concentrations, irrespective of the specific indicator, results in calculated responses at PM concentrations that cannot realistically be controlled. The choice of the model has a dramatic impact on estimated cumulative mortality, which is nearly three times higher for the nonthreshold model than for the threshold model.

Without question, there is need for mechanistic information on the linkage between various PM indicators and health outcomes to guide future revisions of the

PM standard and, most importantly, to guide strategies to control the most hazardous PM. The present PM₁₀ standards and proposed new PM_{2.5} standards are not chemical specific. All particles, irrespective of their chemical composition (e.g., soil dust or combustion emissions), are considered to be of equal toxicity per unit mass. Further, all particles collected within the specified size fraction are considered to be of equal toxicity per unit mass, irrespective of whether the mass represents a few 1-µm diameter particles or a thousand times as many 0.1-µm diameter particles. The large uncertainties in our current knowledge emphasize the need to more clearly understand the causal mechanistic linkage between PM exposure and health outcomes for future reviews and revisions of PM standards.

Despite the uncertainties noted, the U.S. EPA has proceeded with the issuance in mid-1997 of a new rule for PM NAAQS (14). The new rule establishes two new PM_{2.5} standards: *a*) an annual standard set at 15 µg/m³ based on the 3-year average of annual arithmetic mean PM_{2.5} concentrations from single or multiple community-oriented monitors, and *b*) a daily standard of 65 µg/m³ based on the 3-year average of the 98th percentile of 24-hr concentrations at each population-oriented monitor within an area. The rule retains two PM₁₀ standards: *a*) a 24-hr PM₁₀ standard set at

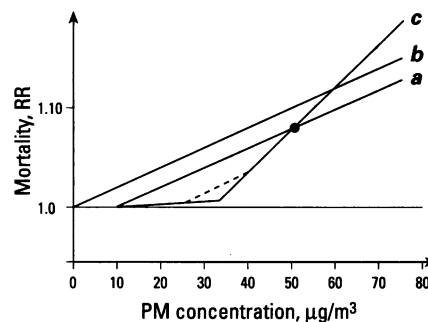


Figure 2. Schematic of alternative interpretations of reported epidemiologic relative risk (RR) findings with regard to possible underlying PM mortality concentration–response functions. *a*, linear model of observed relationship extended to a baseline risk (RR = 1.0) at the lowest observed PM level. *b*, alternative interpretation using the same slope as line *a* extended to essentially zero PM. *c*, an underlying functional relationship with an initial flat segment statistically indistinguishable from the baseline risk (RR = 1.0) until same threshold is exceeded, above which the RR increases. Adapted from the U.S. EPA (6).

65 µg/m³ for the 3-year average of the 99th percentile of the 24-hr concentrations at each monitor within an area, and *b*) an annual PM₁₀ standard set at 50 µg/m³ for the 3-year average of the annual arithmetic mean PM₁₀ concentration at each monitor within an area. The new rule will be reviewed by the Congress and will likely result in substantial debate. Irrespective of their position on implementation of the new standards, it is likely that all parties to the debate will recognize the merits of additional focused research to provide an improved basis for future decisions on the regulation of PM.

Cancer Classification Approaches

Carcinogen classification is the most frequently identified approach; IARC and U.S. EPA schemes are being developed nearly concurrently (13,14). For purposes of brevity, only the IARC scheme will be illustrated (Table 1). Basically, the scheme considers epidemiologic and laboratory animal evidence of carcinogenicity. Since 1991, IARC panels have also evaluated mechanistic data when making a final decision as to classification. An example was the decision to upgrade the classification of ethylene oxide from Group 2A (a probable human carcinogen), where it had been placed based on limited human evidence and sufficient animal evidence), to Group 1

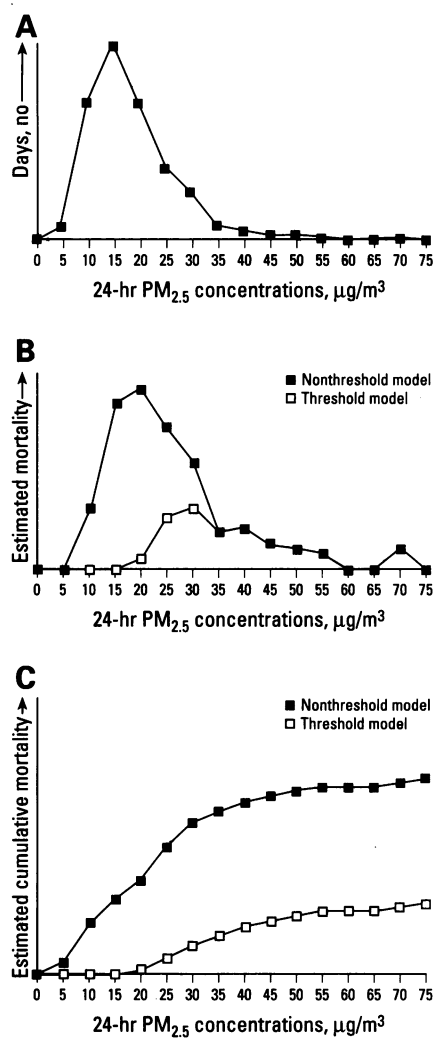


Figure 3. Illustrative air quality distribution of 24-hr PM_{2.5} concentrations showing (A) a frequency distribution of the number of days exceeding various 24-hr average PM_{2.5} concentrations over a year; (B) estimated mortality for nonthreshold and threshold (18 μg/m³) concentration–response relationships; and (C) estimated cumulative mortality for both relationships. Adapted from the proposal for new PM_{2.5} standards [U.S. EPA (7)].

(a human carcinogen) based on mechanistic evidence (15). This created a somewhat awkward situation in that Group 1 now includes two subgroups: a large group of agents for which there is sufficient evidence of human carcinogenicity, and a single agent (ethylene oxide) for which there is limited evidence of human carcinogenicity.

The IARC classification scheme does not address the potency of carcinogens. In short, a carcinogen is a carcinogen, irrespective of its potency. This sharply restricts the use of IARC data in moving beyond what has been termed the hazard

identification stage of risk assessment to a fuller characterization of risk. A full characterization of risk requires information on the potency of the agent as well as an assessment of exposure.

Quantitative Risk Estimation

The third approach used in assessment of PM risk is that of quantitative risk estimation. This approach is most clearly identified with the U.S. EPA. Its use grew out of the agency's need to quantitate the extent of risk posed by various HAP (13,16–18). The quantitative approach has its roots in the assessment of cancer risks with radiation exposure and with the release of radioactive materials from nuclear power industry operations. For radiation exposures, unlike chemical exposures, substantial quantitative dose–cancer response data are available for humans. Unfortunately, neither radiation nor chemical data extend to the low dose (or exposure) rates that are of greatest interest, which necessitates the use of extrapolation models to estimate the risks of low-level exposures. This is the same issue as discussed earlier and illustrated in Figure 2.

Human data that quantitatively relate cancer response to chemical exposure are available for only a few chemicals. Thus two major extrapolations must be made to attempt to quantitate human cancer risk for a majority of chemicals and for most PM exposures: from laboratory animals to humans, and from high levels of exposure to substantially lower levels of exposure.

The U.S. EPA published guidelines for assessing carcinogenic risk (19) and recently proposed major revisions to the guidelines (14). The proposed guidelines take into account many of the suggestions made in the National Research Council (NRC) report *Science and Judgment in Risk Assessment* (17). The guidelines build on the substantial progress made during the last decade in understanding the carcinogenic risks of chemical exposures and how they may be assessed. The proposed guidelines emphasize the value of using specific scientific information on the mode of action of chemicals in risk assessment, using default options only when specific scientific information is not available. A major advance is the guidance for preparation of a narrative statement for each chemical, which summarizes the weight of evidence concerning carcinogenic potential and replaces the alphanumeric classification previously recommended. Another major change is the option of using alternative

models to describe the relationship between low levels of exposures and cancer risk, rather than using the linearized multi-stage model exclusively, as required by the previous guidelines.

The proposed changes will have the greatest impact on assessing risks of the modest number of chemicals for which extensive mechanistic data are available. Examples include formaldehyde, chloroform, and chemicals that produce male rat-specific kidney tumors via an α_{2u}-globulin mechanism (20). With case studies on chemicals such as these, it should be possible over time to extend the approaches to other chemicals. This includes both risk assessment and, of equal importance, the acquisition of new mechanistic information that can reduce uncertainty in assessing risks, as discussed below.

Acquisition of Mechanistic Data

For many years, scientists have attempted to better understand how various kinds of airborne particles cause disease. Often, research has been conducted with a view toward understanding the complex process by which diseases develop and applying the knowledge gained to improve disease prevention, diagnosis, and treatment. In some cases, studies have had a PM orientation, for example, investigation of the characteristics of particles that cause them to vary markedly in their ability to cause disease. To a large extent, a major motivation for past research has been a desire to explore the unknown. This approach to research has led to substantial improvements in our understanding of pulmonary diseases and the ways in which various toxic agents may produce disease. In the past, relatively little research has been explicitly directed toward reducing uncertainties in the assessment of human PM exposure risks. Studies have been conducted at all levels of biological organization, including populations of occupationally or environmentally exposed people, laboratory animals, and studies with isolated tissues and cells. Much of the current research uses the most recent advances in molecular and cell biology.

Our current knowledge of how various kinds of particles and fibers can cause disease can be integrated as shown schematically in Figures 4 and 5 (21). These schemes are for illustrative purposes, with recognition that some linkages are speculative and that not all of the possible steps linking exposure to PM and disease are included. However, it is imperative that we periodi-

cally integrate what we do know about the progression of toxicant exposure—from initial exposure to the dose of toxicant reaching critical molecules and cells and ultimately to the development of disease.

Many advances in our knowledge have come from a reductionist approach that seeks to understand interactions in some small portion of the bigger picture illustrated in Figures 4 and 5. In some cases, investigators have tried to place their findings within the context of these larger scenarios. Unfortunately, the focus in many cases has been on the use of more refined reductionist approaches rather than on adequate consideration of how the observations fit in the larger context, or how the information gained could be used to improve the characterization of human risks from exposure to specific PM.

Over time it is anticipated that an increasingly clear picture will emerge of the mechanisms by which PM may produce disease. However, this may not result in improved risk assessments for PM since all increments of improvement in mechanistic knowledge are not of equal value. Information on the mode of action of specific PM will be of particular value. The mode of action for a toxicant is determined by those key mechanistic steps that link exposure to dose to disease and are crucial in determining the shape of the exposure–response relationship for humans, an approach advocated by Butterworth et al. (22).

Many of the papers presented at this meeting reported findings from studies conducted with only a single exposure or dose level, as contrasted with the long-term human exposures of greatest concern. Further, the exposure (dose) levels studied were frequently extraordinarily high compared with levels likely to be encountered by humans, even under the most extreme of plausible exposure circumstances. Thus, the studies did not yield information readily applicable to understanding exposure (dose)–response relationships for likely human exposures. Many of the studies, even those using the most contemporary molecular approaches, may primarily be providing insight into mechanisms that are unique to the high levels of exposure (dose) studied and may have very limited relevance to understanding the occurrence or absence of disease at likely levels of human exposure. Many of the studies might have yielded information of much greater value if additional lower exposure (dose) levels had been studied.

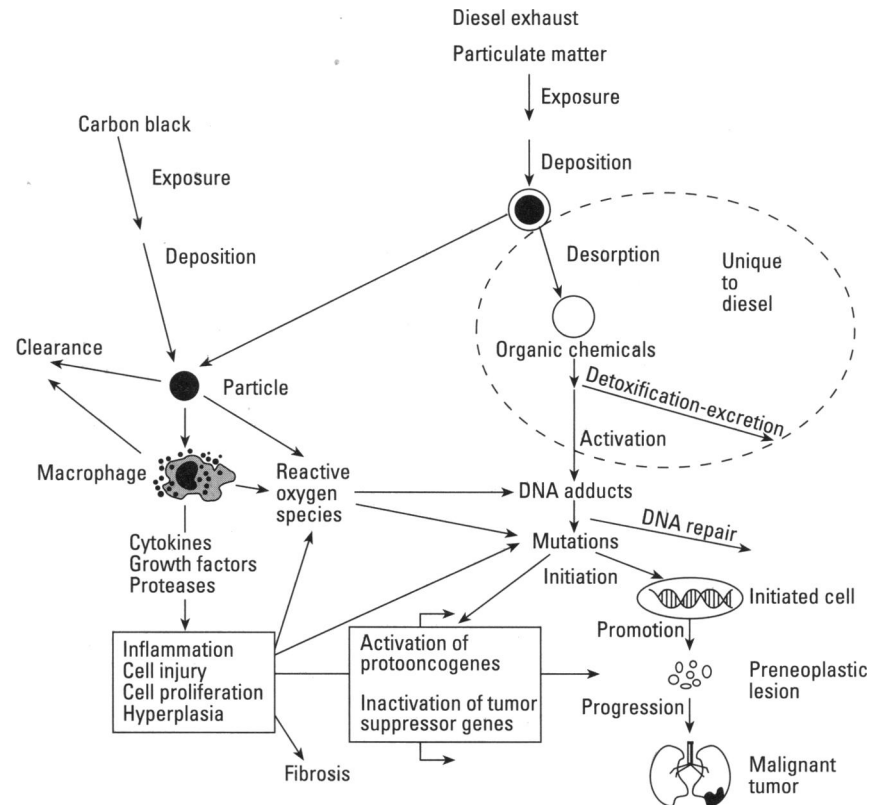


Figure 4. Pathogenesis of lung disease in rats with chronic, high-level exposures to particles.

Understanding mechanisms at lower levels of exposure (dose) is especially important when the primary disease of concern, cancer in this case, has a quantal or dichotomous (i.e., presence or absence of cancer) exposure (dose)–response relationship over the range of exposures intended for extrapolations to lower levels of exposure. For example, our ultimate interest may be in extrapolating from an observed incidence of 1 in 10 to an incidence as low as 1 in 10^6 . For reference purposes, a typical smoker has a probability of about 1 in 10 of dying of lung cancer, with 9 smokers not dying of lung cancer. The dichotomous nature of the response even at high levels of exposure, as in cigarette smokers, is indicative of some of the many mechanistic steps from exposure to the toxicant to the development of disease that also manifest dichotomous rather than continuous responses. Alternatively, some of the individual steps in the multistep process may only be manifest as dichotomous functions when observed at low levels of exposure over long periods of time. Studies conducted at high levels of exposure may result in a saturation of the system, thus masking the dichotomous response.

The cancers of special concern for inhaled particles are typically manifest in increased incidence in people beginning late in midlife. Cancer linked to exposure to toxic agents is usually observed only after a latent period of several decades, and frequently after extended periods of exposure. In contrast, most of the studies reported at the meeting involved only a single brief exposure to a toxicant or exposures over a few days or, rarely, a few weeks.

When exposures are of high intensity and for short periods of time, the events observed largely involve only injury to the system, with repair processes that are totally overwhelmed. The dichotomous nature of the cancer response in a population may be in part a reflection of the interplay between injury and repair at various stages in the carcinogenic process in different individuals. When exposure and time scenarios that minimize the opportunity for repair are studied, what would otherwise be manifest as a dichotomous response shifts to a continuous response function. This complicates the extrapolation of findings to the long-term, low-level exposures that are of concern for human populations.

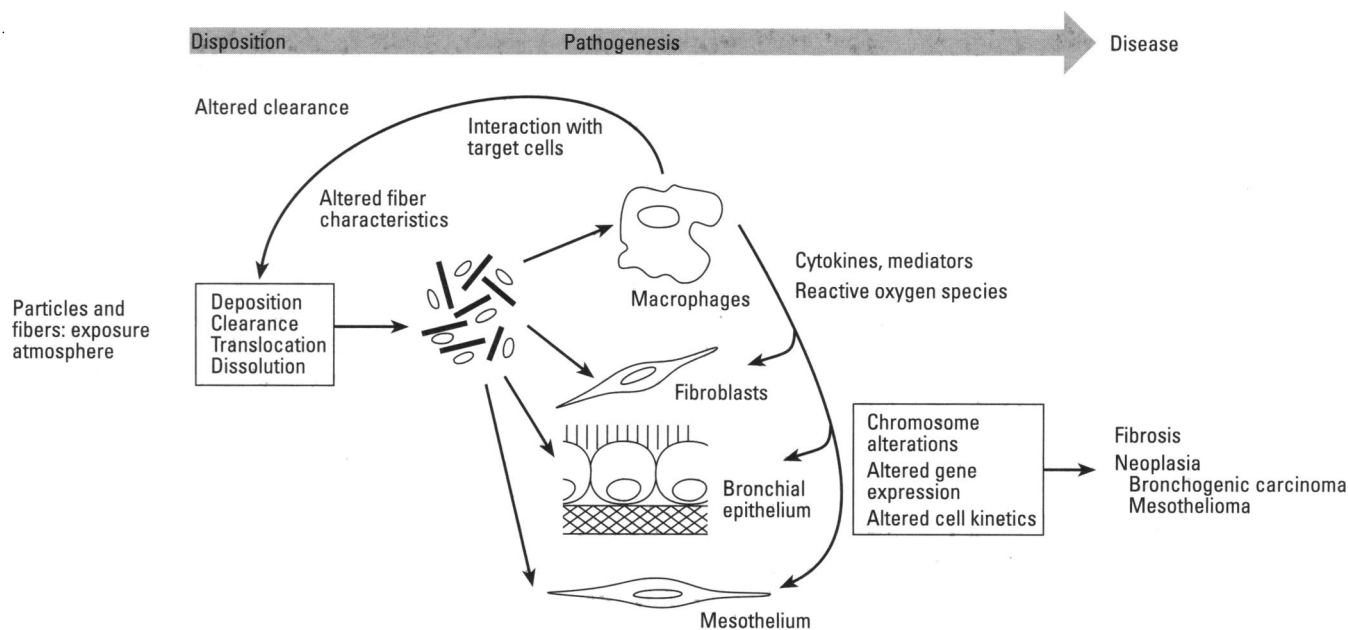


Figure 5. Mechanisms of pulmonary disease induced by chronic high level exposure to highly biopersistent fiber. Adapted from McClellan and Hesterberg (27).

An additional point deserving mention is the role of *in vitro* studies in the acquisition of information that can be used to improve human risk assessments. *In vitro* studies are one of the cornerstones of a reductionist approach to acquiring new insight into complex biological processes such as the effect of inhaled particles on humans. Moreover, the use of *in vitro* systems may minimize the need for *in vivo* studies in laboratory animals.

One advantage of *in vitro* systems is that they can be used to study biological or pathobiological processes in isolation from the more complex milieu of the intact mammalian body. Ultimately, however, the *in vitro* findings must be placed within the context of that more complex *in vivo* milieu. One approach to placing the *in vitro* observations in context is to conduct closely linked *in vitro* and *in vivo* studies. Another useful approach is the use of coculture systems that utilize epithelial cells cultured both with and without macrophages. Such linkages were apparent for a few of the papers presented at this meeting. Unfortunately, observations made *in vitro* with highly sophisticated experimental techniques were not linked to the *in vivo* situation in many cases.

In *in vitro* studies, there is a special need to establish the relevance of the exposures (doses) to possible results *in vivo*. With *in vivo* studies, experimental limitations impose upper bounds on the quantity of particles that can be delivered to the cells of

the respiratory tract. In working with *in vitro* systems or in *in vivo* studies with instillation or injection of materials, these experimental limitations are removed, and it is possible to deliver to cells quantities of particles that are many times greater than could be found in the body under any plausible human exposure scenario. The researcher working with *in vitro* systems is urged to perform calculations that illustrate the relationship between the doses used *in vitro* to those likely to be found *in vivo* in laboratory animals and, ultimately, to make comparisons with plausible human exposure. When particles are delivered by nonphysiological modes such as intratracheal instillation to instantaneously achieve a large lung burden of PM, it is important to recognize that the effects may differ from a slowly accumulating burden of PM.

The last point to be made before concluding this discussion of past mechanistic approaches is the need to remember that our ultimate interest is in understanding and assessing human health risks from exposure to airborne PM. This requires that *in vivo* studies with laboratory animals and *in vitro* studies with tissues and cells from laboratory animals be related back to humans. One approach is to conduct *in vitro* studies with both human and laboratory animal tissues and cells, further clarifying the interpretation of findings from laboratory animal *in vivo* studies. Studies with human tissues, when practical, should include a sufficient number of samples to get a sense of the range and

variability of key parameters in human populations. An additional approach is to study multiple laboratory animal species to identify both similarities and differences among laboratory species. This approach provides an improved basis for evaluating the likely nature of the human response. Several papers presented at this meeting illustrated clear differences in the responses of different species to inhaled particles in the respiratory tract. A better understanding of the basis for both interspecies differences and similarities in exposure-response relationships will enhance our ability to estimate human risks of PM exposure.

Use of Mechanistic Data

I have just argued that many of our high dose, short-term mechanistic studies exaggerate injury and discount repair and shift what might be dichotomous responses to continuous responses. This assertion has important implications for the use of such studies in those carcinogen classification schemes that are placing increased emphasis on the use of mechanistic data. Both IARC and the NTP recently opened the door to increased use of mechanistic data to either upgrade or downgrade the carcinogen classification of individual chemicals. IARC already upgraded ethylene oxide from Category 2A (probable human carcinogen) to Category 1 (a human carcinogen) based on mechanistic data in the absence of sufficient evidence of human carcinogenicity. The NTP (23) has proposed classifying

agents as “reasonably anticipated to be human carcinogens” based on mechanistic data in the absence of evidence of carcinogenicity from animal bioassays.

These approaches to incorporating mechanistic data into the carcinogen classification process have the potential for classifying agents as carcinogens that are only high-dose laboratory animal carcinogens, only high-dose carcinogens in a single laboratory animal species, or agents that at high doses produce some preneoplastic mechanistic responses but have not been evaluated as to their carcinogenicity in whole-animal studies. Carbon black, a high-dose rat carcinogen, is illustrative of the second type of agent.

The next edition of the NTP *Biennial Report on Carcinogens* will include the first agents classified as “reasonably anticipated to be human carcinogens” based on mechanistic data in the absence of direct animal evidence of carcinogenicity. Most of the agents initially considered for listing are combustion products. Major controversy is likely to arise when the first commercial agent is listed as “reasonably anticipated to be a human carcinogen” in the absence of direct animal or human evidence. With regard to carbon black, long-term exposure of rats to high concentrations causes an excess of lung cancer in rats compared to controls (24,25). The studies of Driscoll (26), Driscoll et al. (27), and Oberdörster (28) provide clear evidence of a mechanism of action for carbon black producing lung cancer despite the fact that carbon black does not interact directly with DNA to produce mutations. The mechanism involves chronic high-level exposure to carbon black to produce persistent inflammation, with associated high levels of cytokines and mediators, and an increased frequency of mutations.

An IARC panel considered the two positive rat bioassays to be sufficient evidence of animal carcinogenicity; despite the mechanistic information for a high-dose, rat-specific mechanism, IARC classified carbon black in Category 2A (a probable human carcinogen) (29). I previously indicated a line of reasoning that would have placed carbon black in Category 3 (not classifiable). The IARC panel apparently was of the opinion that the currently available data did not prove that small particles cannot cause cancer in humans. In reaching this conclusion, the panel no doubt considered quartz and asbestos, which do not interact directly with DNA and are classified by IARC as human carcinogens.

In considering the carcinogen classification issue for carbon black, I noted the value of augmenting an alphanumeric classification scheme with a narrative statement (30). In *Science and Judgment in Risk Assessment* (17), the Committee on Risk Assessment of Hazardous Air Pollutants recommended the use of a narrative statement describing the evidence for an agent's carcinogenicity in humans. The U.S. EPA followed this recommendation in the proposed revision of its carcinogen risk assessment guidelines (14).

In addition to the issues associated with classifying agents as to their carcinogenicity, the carcinogenic potency of an agent should be established in quantitative or semi-quantitative terms. Present carcinogen classification schemes do not consider either the likely potency of an agent or current and anticipated levels of human exposure. Because potency is not considered, agents that differ markedly in their potency may be placed in the same category. Proponents of present classification schemes frequently argue that these schemes are intended only to provide input into the hazard identification phase of risk analysis. This is a short-sighted view, which results in less available information for government and private officials, workers, and the public at large to use for input into decisions on the best way to deal with possibly carcinogenic agents.

Suggestions for a Change in Approach

The use of formalized risk analysis has increased markedly during the past two decades, and the methods used for risk analysis have continually evolved. These

changes are readily apparent from consideration of the 1983 NRC report, *Risk Assessment in the Federal Government: Managing the Process* (31); the 1994 NRC report, *Science and Judgment in Risk Assessment* (17); the 1986 U.S. EPA *Guidelines for Carcinogen Risk Assessment*; and the 1996 U.S. EPA proposed revisions to the 1986 guidelines (14). Two inter-related themes are apparent from a review of both sets of documents. The first theme relates to uncertainty and the need to characterize it for use in future research. Such feedback should reduce uncertainty in future risk assessments. The need to characterize uncertainty, and the opportunity to reduce it as risk assessments are carried out in an iterative fashion, are dominant themes of the 1994 NRC report. The use of the feedback loop, from risk assessment to the research arena, is schematically depicted in Figure 6.

A second closely related theme is the use of specific scientific information to replace default options. Default options are selected to be conservative (i.e., more likely to overestimate than to underestimate risk); thus the introduction of scientific information specific to the risk assessment being conducted should serve to reduce uncertainty in the specific assessment and is also likely to reduce the estimated risk.

The acquisition of mechanistic information is central to both themes. New information, including mechanistic information, will have maximum impact on the risk assessment process when it is relevant and usable in the process. In earlier sections, I raised questions concerning the relevance of some of the mechanistic information obtained in the past. Information

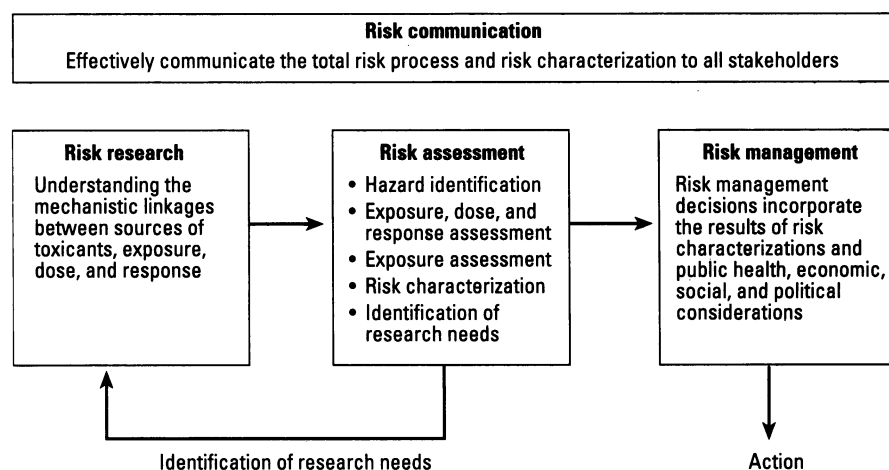


Figure 6. Risk assessments can help identify research needs that, if addressed, can reduce uncertainty in assessing risks.

that is of limited relevance for assessment of human risks may in fact increase uncertainty in the estimation of human risks.

To increase the usefulness of information obtained from future research directed toward reducing uncertainty in assessment of human health risks from exposure to particles, I suggest use of the following guidelines:

- Mechanistic studies should be prospectively designed with a clear view as to how results of a given study will be used in the risk assessment process. This can be done by stating experimental objectives in the form of testable hypotheses that are linked to the risk assessment process (Figure 7). In many cases, these hypotheses will address issues related to default options that would otherwise be used in the absence of specific scientific data.
- Exposures (doses) to be used should be evaluated to establish their relationship to plausible levels of human exposure and, for *in vitro* studies, to tissue levels likely to be observed in *in vivo* studies using realistic exposure levels. Implementation of this guideline might be facilitated by establishing maximally tolerated doses (U.S. EPA perspective) or minimally toxic doses (European Economic Community perspective) for *in vitro* studies analogous to those advocated for chronic toxicity and carcinogenicity studies. This issue, as relates to *in vivo* studies, has been extensively reviewed (31–33).
- In the conduct of *in vitro* mechanistic studies, use three or more dose levels of the toxicant in addition to a control group when practical, to achieve a gradient of response relative to dose. The goal of such studies should go beyond demonstrating an effect and its underlying mechanism and extend to elucidating

how the mechanism or mechanisms are influenced by dose and dose rate.

- When conducting short-term *in vivo* studies of mechanisms of action, investigators should use three or more exposure levels to achieve a gradient of response relative to exposure. The highest exposure level should be no higher than the highest exposure likely to be tolerated in a 90-day exposure study; the lowest level should be the lowest practicable exposure that yields tissue doses equivalent to the highest levels likely to be observed in humans. The goal of such studies should be similar to that noted in the previous guideline.
- When practical, observations made *in vitro* with tissues or cells obtained from laboratory animals should be extended to human tissues or cells. When practical, samples from a sufficient number of individuals should be evaluated to characterize interindividual variability.
- When practical, the result of mechanistic studies should be presented quantitatively within the framework of an exposure (dose)–time–response matrix rather than reported as yes–no phenomena.
- When interpreting mechanistic data for use in carcinogen classification schemes or other aspects of risk assessment, investigators should place substantially greater weight on data obtained over a range of exposure (dose) levels and longer periods of observation. Less weight should be given to observations made at a single high exposure (dose) level and with short periods of observation.
- Mechanistic data should be used for risk assessment purposes only when they are more likely to represent a mechanism operative in humans at plausible levels of exposure.

Ultimately, whatever mechanistic data are developed must be integrated with the results of animal bioassays and whatever human data are available to provide a weight-of-the-evidence assessment of human risk. The evaluation and integration process for all of the various pieces of information is complex and obviously involves considerable judgment. Hill (34) provided valuable guidance for consideration of epidemiologic evidence of the relationship between environment and disease and, specifically, whether the evidence was one of association or causation. The nine viewpoints he related are of considerable merit for all types of evidence, not just epidemiologic, in assessing human risks of

exposure to agents present in environmental or occupational settings. Hill's viewpoints (34) are paraphrased below:

- Strength: What is the strength of the relationship between the agent or an individual event in the pathogenesis of the disease and the disease?
- Consistency: Has the association been observed by different investigators, in different types of studies, in both human and laboratory animal tissues?
- Specificity: Is the association specific to a particular PM or class of PM, or is it a general aspect of the disease process not unique to any particular agent? Is the mechanistic event obligatory with regard to development of disease?
- Temporality: Where does a particular pathogenic event fit into the linkage between exposure to PM and occurrence of disease? Does it occur early or late in the disease process?
- Biological gradient: Is a biological gradient or an exposure (dose)–response relationship observed? What are the lower limits for statistically detecting evidence of an increase in some mechanistic event versus increasing exposure (dose)?
- Plausibility: Do the data indicate biological plausibility? In considering plausibility, it is important to recall Hill's admonishment—"What is biologically plausible depends upon the biological knowledge of the day."
- Coherence: Is a specific observation supported or reinforced by other observations?
- Experiment: What is the experimental evidence? In evaluating the role of a specific agent causing a series of mechanistic events, have adequate positive and negative controls been experimentally evaluated?
- Analogy: Are the same or similar mechanistic linkages observed with other similar PM exposures?

In my opinion, Hill (34) was advocating what we now call a weight-of-the-evidence approach in which all of the evidence is considered, as contrasted to a strength-of-the-evidence approach that emphasizes the use of positive studies. The use of this kind of robust approach to risk assessment is needed since new materials with great potential value to society will continually be discovered and commercialized. It is imperative that we improve the approaches available for evaluating the potential risks of these new materials and the processes by which they are produced prior to introducing them into commerce.

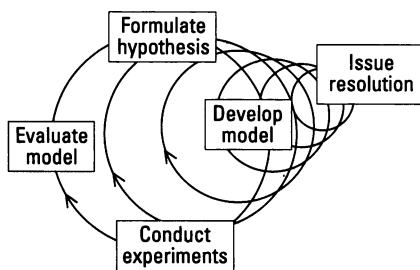


Figure 7. Iterative approach to formulation of hypotheses, development of models, and conduct of mechanistic research to improve risk assessments.

At the same time, there is a need to govern against using systems that have potential for yielding an excess of false-positive results that may exclude potentially valuable products from commerce.

The above guidelines are not intended to be either exhaustive or constraining. They are offered to stimulate discussion as we attempt to collectively bring more and better science to bear in the assessment of

human health risks from exposure to a wide range of agents.

Summary

In this article, I briefly reviewed the approaches used to assess human health risks of PM exposures and the role of mechanistic data in improving the process. Guidelines for the development and use of mechanistic data in the risk assessment

process are offered to stimulate discussion of approaches for better links between the acquisition and use of mechanistic data in the risk assessment process. In doing so, we need to expand the statement of Paracelsus (1493–1541): “What is there that is not poison? All things are poison and nothing (is) without poison. Solely the dose and the mechanism of action determine that a thing is or is not a poison.”

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