PROPOSE HEALTH EFFECTS TEST GUIDELINES FOR RESPIRABLE FIBROUS PARTICLES

David Lai
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, DC

Abstract

The current EPA health effects test guidelines for chronic inhalation toxicity and/or carcinogenicity studies are not specific enough for the testing of fibrous substances which have been identified as priority substances for risk reduction and pollution prevention due to potential health concern. Thus, there is a need for EPA to develop standardized health effects test guidelines for fibrous substances that can be used by EPA to obtain the necessary toxicologic information for risk assessment. On July 28, 1999, EPA published the proposed combined chronic toxicity and carcinogenicity guidelines for testing fibrous particles. The proposed test guidelines were developed based on the guidance provided by an expert panel specific for the design and conduct of chronic inhalation studies of fibers in rodents.

Introduction

Natural and synthetic fibers are one group of substances that have been identified to be of potential health concern. Many of these fibers have wide industrial and commercial applications, and there are limited, inconclusive, or virtually no information about their health effects and/or exposure to workers, consumers, and the general public. As a result, the U.S. Environmental Protection Agency (EPA) has added a "respirable fibers" category as priority substance for health effects and exposure testing to obtain the necessary data to evaluate the extent and magnitude of health risks to exposed individuals and populations.

The health concern for respirable fibers is based on the link of chronic inhalation exposure to asbestos and erionite fibers to the development of respiratory diseases, including cancer in humans. There is also experimental evidence showing fibrogenic and carcinogenic effects in laboratory animals exposed to a variety of fibers of varying physical and chemical characteristics. The mechanisms by which mineral fibers cause fibrogenic and carcinogenic effects in humans and animals are not clearly understood. However, there is extensive evidence relating fiber size, lung disposition and clearance, and bioavailability to fiber toxicity and carcinogenicity. The toxic potential of a fiber appears to be dependent on the respirability and the nature of the fiber. Therefore, fibrous particles of respirable sizes and can survive in biological systems for long periods of time are considered of potential hazard concern.

EPA recognizes that the current health effects test guidelines for chronic inhalation toxicity and/or carcinogenicity studies on chemicals are not specific enough for the testing of fibrous substances. These guidelines have to be modified to take into account testing issues which are unique to fibrous particles. Although a number of test systems and/or protocols (e.g., WHO, 1992; ISTR, 1994) have been utilized by the scientific community for evaluating the fibrogenic and carcinogenic potential of fibrous particles, there has been considerable debate about the scientific validity and utility of available test methods. Thus, there is a need for EPA to develop standardized health effects test guidelines for fibrous substances that can be used by EPA in future rulemaking, negotiated enforceable consent agreement, or voluntary action to obtain the necessary toxicologic information for risk assessment.

Major Issues Related to Fiber Testing

On May 8-10, 1995, EPA, in collaboration with the National Institute of Environmental Health Sciences (NIEHS), the National Institute for Occupational Safety and Health (NIOSH), and the Occupational Safety and Health Administration (OSHA), convened a panel of expert scientists at a workshop to evaluate a number of scientific issues related to chronic inhalation toxicity and carcinogenicity testing of respirable fibrous particles (Vu et al., 1996; EPA, 1996). Major issues for discussion were on: (i) the optimal design and conduct of studies of the health effects of chronic inhalation exposure of animals to fibers; (ii) preliminary studies which would be useful guides in designing the chronic exposure study; (iii) mechanistic studies which would be important adjuncts to the chronic exposure study to enable better interpretation of study results and extrapolation of potential effects in exposed humans; and (iv) available screening tests which can be used to develop a minimum data set for (a) making decisions about the potential health hazard of the fibers, and (b) prioritizing the need for further testing in a chronic inhalation study.

After extensive discussion and debate of the workshop issues, the general consensus of the expert panel is that chronic inhalation studies of fibers in the rat are the most appropriate tests for predicting inhalation hazard and risk of fibers to humans. The followings are some of the conclusions and recommendations made by the panel at the workshop that have been incorporated into a proposed health effects test guidelines for the design and conduct of chronic toxicity and carcinogenicity inhalation studies of fibers in rodents (EPA, 1999).

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- A fiber is defined as a particle having an aspect ratio of at least 3:1 (length : diameter) and being structurally continuous. A "rat-respirable fiber" is defined as a fiber having an aerodynamic diameter of less than 3 μm.
- There are considerable differences in fiber inhalability and respirability between humans and laboratory rodents. This observation raises several questions with respect to the choices of fiber samples to be tested, recognizing the inherent limitations of using rodent species as surrogates of humans in inhalation studies, and the need for optimizing the study conditions while still being able to obtain pertinent toxicologic information for extrapolations to humans. To maximize sensitivity of animal inhalation exposure studies to health effects of fibers, the test material should consist of rat-respirable fibers and should be enriched with the most potent human respirable fraction (*i.e.*, long, thin fibers; aerodynamic diameter less than 3 μm, and lengths at least 20 μm or fibers with high aspect ratios).
- There is considerable evidence to suggest the importance of fiber characteristics in relation to disease outcomes. Thus, it is desirable to obtain data on a number of the physical and chemical properties of the particles. These data will also enable the investigator to make some preliminary estimates of the lung burden of the material at a given exposure concentration, the behavior of the particle in the lung, and to some extent, its expected toxicity. The following physicochemical properties of the test samples should be provided: fiber morphology, dimension, size distribution, aerodynamic diameter, chemistry, density, solubility, surface characteristics, the ability of a fiber to split longitudinally or cross-sectionally.
- The complete bivariate length and diameter distribution should be determined in the aerosol and in the lung via electron microscopy (SEM/TEM). Routine monitoring to control the day-to-day aerosol concentration can be performed using phase-contrast optical microscopy (PCOM) and gravimetric techniques. The contribution of non-fibrous particulate materials should be assessed since they may be substantial and could add significantly to total lung burden in terms of mass.
- Inhalation studies with asbestos fibers in rats have been demonstrated to be appropriate experimental models for the identification of asbestos-induced human diseases, primarily fibrosis and cancer of the lung. Chronic inhalation studies of fibers in the rats are the most appropriate tests for predicting inhalation hazard and risk of fibers to human. The hamster appears to be more sensitive than the rat with respect to fiber-induced mesothelioma. When mesothelioma is the endpoint of concern, testing in the hamster as a second species is recommended.
- Both sexes of rodents should be used because data on sex differences in response to inhalation exposure to fibers are limited.
- There are advantages and disadvantages associated with either method of exposure- whole body exposure and nose-only exposure. Either nose-only or whole-body exposure can be used.
- Along with other information (decrease in body weight, systemic toxicity, etc.), data should be obtained on lung burdens and bronchoalveolar lavage fluid (BALF) analysis in a 90-day subchronic inhalation study to assist in establishing the chronic exposure levels. The MAC (Maximum Aerosol Concentration) should be based on a combination of the following parameters: altered alveolar macrophage mediated particle clearance rate, fiber lung burden normalized to exposure concentration, cell proliferation, histopathology, inflammation (quantitatively determined as percentage increase in polymorphonuclear leukocytes [PMNs] in lung lavage samples) and lung weight.
- For the chronic study, three exposure concentrations should be used; the high exposure concentration and resulting lung dose should show significant effects in the above parameters, and the lower doses should be appropriately spaced and be selected based on results from the 90-day study and from previous studies with the particular fiber.
- The chronic inhalation exposure study with fibers should be a lifetime study, with exposure terminated at 24 months in rats and the study terminated when survival of the control group reaches 20%. Due to the shorter lifespan of hamsters, their exposure duration could be shorter, i.e., 18 months.
- Interim sacrifices are essential and should be made at 3, 6, 12, 18, and 24 months in rats. The endpoints evaluated at these times should be the same as in the subchronic study. Data obtained from lung burden analysis can be used not only for establishing the chronic exposure levels or aerosol generation changes needed to get more fibers deep into the lung, but also for the quantify aspects of risk assessment related to dosimetric adjustments before extrapolation. Bronchoalveolar lavage fluid (BALF) analysis should be required in the chronic study. BALF analysis will not only enable the investigator to better select the exposure concentrations for the chronic studies but also to

help in understanding the biochemical and cellular sequence of events of particle-induced toxicity and carcinogenicity. Impairment of clearance should be assessed via challenge with a tagged particle.
- A positive control need not be included in every study, but each new test system (including use of a different animal species or strain) should be validated with a positive control material.
- Neoplastic endpoints recorded should include epithelial hyperplasia, alveolar bronchiolization, metaplasia, adenomas, mesotheliomas, and carcinomas.

The workshop panel also concluded that no single assay or battery of short-term assays can predict the outcome of a chronic inhalation bioassay with respect to carcinogenic effects of fibers. However, it was suggested that appropriately designed Tier II (*in vitro*) and Tier III (short-term *in vivo*) studies – as defined in an earlier workshop sponored by the Chemical Industry Institute of Toxicology (McCllellan et al., 1992) -- can provide useful information to assess the relative potential of fibrous materials to cause toxicity in the lung and associated tissues. Along with information on physicochemical properties, data from a battery of short-term *in vitro* solubility/durability and cell toxicity assays (Tier II), and short-term inhalation studies (Tier III) can be used to screen and set priorities for further chronic testing of fibers.

Summary

The EPA's current health effects test guidelines for carcinogenicity, and combined chronic toxicity and carcinogenicity are widely accepted by the scientific and regulatory communities for the testing of chemical substances (EPA, 1998). It is recognized, however, that these guidelines need to be modified to take into account testing issues which are unique to fibrous particles. On July 28, 1999, EPA proposed the combined chronic toxicity and carcinogenicity test guidelines for use in the testing of fibrous particles(EPA, 1999). The proposed guidelines were developed based on the comments and recommendations made by a workshop panel experts on a number of scientific issues related to fiber testing (EPA, 1996). Whereas no single assay or battery of short-term assays can predict the outcome of a chronic inhalation bioassay with respect to carcinogenic effects, data from a battery of *in vitro* and *in vivo* short-term studies can be used to screen and set priorities for chronic testing of fibers.

Disclaimer

This paper has been reviewed and approved by the Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency. Approval does not signify that the contents necessarily reflect the views and policies of the Agency.

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