BSC Nomination Review

Meeting of the National Toxicology Program
Board of Scientific Counselors

National Institute of Environmental Health Sciences
Rall Building, Rodbell Conference Center
Research Triangle Park, NC

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NTP Study Nomination:	Naturally ocurring asbestos and related mineral fibers	
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1. **Does the NTP research concept address the needs of the nomination?**

This NTP Nomination proposes to study the mineralogy, morphology, biopersistence, and carcinogenicity of naturally occurring asbestos (NOA) and other atypical forms of asbestos fibers. NOA is widely distributed throughout the United States and occurs in natural deposits as mixtures of asbestiform fibers, acicular crystals, and cleavage fragments together with other minerals including calcite and quartz. Some of these asbestiform fibers have been characterized as amphiboles that are currently not covered by regulations applied to commercial amphiboles. The recent public health tragedy at Libby, Montana has raised concerns about environmental and community exposures to NOA.

The NTP Nomination proposes a research strategy to address the following uncertainties and data gaps:

- What are the potential human health effects of NOA?
- What are the dose-response relationships especially focusing on low-level, continuous exposures in the community?
- What is the relative potency of these diverse mineral structures and shapes?
- What are the physical and chemical properties of NOA related to toxicity and carcinogenicity?

Rationale for this NTP Nomination

Concerns have been raised by ATSDR (Expert Panel Report, 2003) and US EPA about adverse human health effects of NOA. It is urgent to assess the potential adverse health effects of other NOA in order to prevent future asbestos-related diseases in El Dorado County, California and other sites world-wide where community and environmental exposure to NOA

may occur. Community and environmental exposureto asbestos affects men and women equally and raises concern about future health impact on children who are exposed beginning at an early age.

Several nonmalignant and malignant disease have been associated with exposure to asbestos fibers:

- asbestosis or diffuse interstitial fibrosis of the lungs
- bronchogenic carcinoma
- cancer of the larynx (IOM Report, 2006)
- nonmalignant disease of pleura:
    i. pleural plaques
    ii. diffuse visceral pleural fibrosis
    iii. pleural effusions
- malignant pleural and peritoneal mesothelioma

Asbestosis, bronchogenic carcinoma, and nonmalignant and malignant pleural diseases have been diagnosed among the residents of Libby, Montana. Some of these cases occurred in workers employed at the vermiculite mine and mill; other cases occurred in households of workers and other residents in the community. Malignant mesothelioma and pleural plaques can develop after lower levels of exposure than asbestosis or bronchogenic carcinoma; therefore, these nonmalignant and malignant diseases are of concern for community and environment exposure to NOA, especially the amphiboles that contaminate the vermiculite mine in Libby, Montana. According to an ILSI Working Group Report published in *Inhalation Toxicology* in 2005: *At this time, no single physicochemical property or mechanism has been defined that can be used to predict carcinogenicity of all fiber types.* It is not clear which physical and chemical properties are relevant to the development of these different nonmalignant and malignant asbestos-related diseases.

There is an urgent need for additional research to characterize and explore potential toxicity and carcinogenicity of NOA, and the proposed research concept generally addresses the needs of the nomination. There are specific aspects of the research program that should be considered before implementation, and these are outlined in detail below.

2. **Is the proposed study approach as outlined in the research concept document appropriate in scope given the merit of the nomination? Are there other studies that should be considered for this substance?**

This is an ambitious, and very expensive program. It will conduct what appears to be the most comprehensive and well controlled animal safety assessment of mineral fibers conducted to date. This program will require significant NTP resources and will probably cost as much as 3-6 times the cost of a more conventional chemical evaluation program. This cost is warranted based on the significant human exposure to these materials. In addition, in spite of the decades of research on asbestos, we still know surprisingly little about the actual mechanism of action of asbestos-induced carcinogenicity and these studies may provide valuable insights into this activity of mineral fibers.

The exact nature of the studies proposed are not described in detail so it is unclear what endpoints are to be included and what special studies will be conducted in conjunction with this program. Recommendations for studies or endpoints to be considered (or dropped) under this program are listed below:

1. To collect and characterize representative samples of NOA.

These initial studies will focus on the Libby vermiculite mine and will be expanded to include ferroctinolite from Minnesota and amphiboles from El Dorado County, California. Erionite fibers from Oregon or South Dakota may also be included. The physical and chemical properties of these fibrous minerals will be compared with a standard commercial amphibole asbestos sample, ideally one that has been tested previously in a chronic rodent inhalation assay. The possibility of including a negative reference fiber sample such as wollastonite was also discussed. The SAB board noted that the techniques required for complete and reliable characterization of asbestos fibers, especially those on the nanoscale, are not available. It was also noted that surface chemistry and surface reactivity should be assessed because these properties cannot be predicted from the bulk chemistry. Physical chemical expertise (e.g., Professor Bice Fubini at the University of Turin or Dr. Ronald Mason at NIEHS) will be required for these studies, in addition to the proposed mineralogic characterization. The potential for additive or synergistic effects of exposure to mixtures of NOA with other minerals such as talc, vermiculite, clay, calcite, and crystalline silica should be considered. The potential for alteration of fiber properties during milling and processing of vermiculite ore should be investigated.

2. In vitro durability and cytotoxicity studies.

These studies are part of a tiered testing strategy as proposed by the ILSI Working Group, 2005 for manmade fibers. These tests could be used to prioritize samples for more expensive in vivo inhalation studies. Acellular durability assays are proposed to test these NOA samples. This is an important assay; however, because these NOA samples are of the amphibole mineral family, it is highly likely that they will be durable in these tests. Even chrysotile asbestos is more durable than certain manmade vitreous fibers in these acellular assays. In vitro cellular assays can be used for mechanistic studies and it is very important to determine whether NOA fibers are mutagenic and/or clastogenic. However, the ILSI Working Group raised concerns about short-term in vitro cytotoxicity studies:

> *There are several issues that limit the usefulness of in vitro tests for toxicity screening of fibers. For example, short-term in vitro assays of biological activity cannot allow for differences in biopersistence of fibers, and as a result, some nonbiopersistent fibers (e.g. glass fibers) that are not pathogenic in vivo are positive in short-term in vitro tests.*

In addition, these are several technical concerns regarding current in vitro cellular toxicity assays:

- Difficulty in extrapolating endpoints based on high-dose, short-term exposure in vitro to low-dose, chronic exposure in vivo.
- In vitro endpoints are short (hours or days) and have not been validated as predictive of chronic diseases that develop over decades in a complex, multicellular environment.
- Primary cell cultures should be used for cytotoxicity assays but these cultures are highly variable and not standardized.

The SAB panel members commented that NTP should develop strategies to improve short-term cellular toxicity assays (e.g., co-cultures, three-dimensional models of tissues, development of predictive biomarkers) that would be more suitable to evaluate NOA. It is especially important to assess direct vs. indirect mechanisms of genotoxicity as well as the interrelationships between persistent inflammation, cell proliferation, fibrosis, and cancer.

In summary, because of the limitations in the ability to extrapolate in vitro results with fibers to in vivo predictions, the inclusion of in vitro studies should be carefully evaluated before proceeding with this portion of the research program.

3. Short-term in vivo assays to assess biopersistence and noncarcinogenic endpoints following 5-14 day inhalation exposure in rats.

These studies are important and feasible, as long as technical issues related to sample preparation, aerosolization, and dosing are addressed (refer to ILSI Working Group Report, 2005). It is very important to include complete fiber and particle characterization of both lung and pleural dust burdens; techniques for pleural dust burdens have been described by Gelzleichter et al. (formerly at CIIT). Additional subchronic endpoints for lung and pleural inflammation, target cell proliferation, fibrosis, and histopathology should be included in comparison with positive and negative reference fiber samples. A major caveat in these proposed short-term assays is the likelihood that amphibole NOA samples will be biopersistent and these rodent inhalation assays will not be able to rank these different samples based on lung fiber burden and biopersistence. An important alternative to consider (raised previously by Drs. Bruce Case and James Lockey and PETA) is to determine lung and pleural fiber and dust burdens at autopsy of the residents of Libby, Montana (see comments from ATSDR Expert Report and meeting notes in 2003).

4. Chronic rodent inhalation assay for carcinogenicity.

A 90-day nose-only inhalation exposure followed by lifetime observation in rats has been proposed by NTP. It is important to extend this exposure for at least one year as discussed by Dr. Eugene McConnell in the ATSDR Expert Report in 2003. Lung cancer and malignant mesothelioma develop late in life of rodents; if exposure is stopped after 90 days, inflammation and fibrosis may not persist and few rats would develop lung cancer. State-of-the-art lifetime chronic inhalation assays of manmade fibers using chrysotile or amosite asbestos as positive reference samples have been conducted by RCC in Geneva, Switzerland (e.g., Hesterberg et al., 1993, 1999 and McConnell et al., 1999) . In these studies, rats exposed to chrysotile asbestos fibers developed both fibrosis and lung cancer, but only 1/69 rats exposed to chrysotile asbestos

at 10 mg/m^3 developed pleural malignant mesothelioma. As raised by PETA, rats are not the appropriate species to assess relative potency of fibers to induce malignant mesotheliomas, although they do develop lung fibrosis and cancer following inhalation of a variety of fiber types as well as particulate dusts. However, in the RCC studies, 22/85 hamsters exposed to amosite asbestos developed pleural malignant mesotheliomas but no lung cancers. This was a multidose study using amosite asbestos as a positive reference fiber; however, in the high-dose group many of the hamsters developed intestinal problems requiring treatment with antibiotics.

In summary, in the proposed chronic carcinogenicity assay, it is likely that amphibole NOA samples will be biopersistent and induce lung fibrosis and cancer in rats. It will be difficult to rank potency unless a wide range of doses is tested. Hamsters are the appropriate test species for development of pleural mesothelioma, but it will be difficult and expensive to test a wide range of doses in chronic inhalation assays, using two species.

5. Other Considerations

- The need to predict effects in children is critical. We recommend inclusion of some form of juvenile animal studies covering a period similar to that of early childhood to adulthood in humans. These need not be full multi-generational studies as have recently been conducted by the NTP with genistein and other compounds, but a thorough evaluation of effects in young (e.g. weanling to sexual maturity) animals is warranted.
- Mechanistic endpoints should be incorporated into both short and long-term exposure studies in animals. For example, cell proliferation (e.g., via PCNA) should be evaluated in several key target tissues. Other inflammatory endpoints (e.g., cytokine levels) should also be evaluated.
- Because asbestos is widely considered to be "non-genotoxic," some form of an in vivo genotoxicity endpoint in an appropriate target tissue (e.g., transgenic mutation in mouse lung) should be considered for at least the short-term (14 or 90-day studies).

3. **Does the proposed research program address an important area of biomedical research (e.g. children's health, genetic susceptibility, specific environmental disease) and/or advance the field of environmental health sciences?**

This program will address a very significant problem in biomedical research, environmental disease and in public health. Despite the widespread past use of asbestos, and the continuing human exposure in some areas and occupations, we still have a poor understanding of the relative hazards of different fibers based on chemical composition, size, exposure regimens, and other factors. In particular, addressing children's health (See #2 above) should be an important consideration in this program.

4. **Do the nomination and proposed research program merit NTP evaluation and if so, what priority (low, moderate, or high) should it be given?**

The response to this question is dependent on the relative merits of other programs being considered by the NTP. Given the substantial human exposure, the significant exposure of children, and the relative lack of understanding of asbestos-induced disease mechanisms, I would

give high priority to this program. In contrast, conduct of this program will probably use budget and other resources that will prevent several other compounds from being evaluated by the NTP. Therefore, while I consider this program to be worthy of funding, one must consider the relative merits of this program vs. the programs that will be cancelled or postponed because of this work.

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