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Health Effects Test Guidelines: OPPTS 870.8355 Combined Chronic Toxicity/Carcinogenicity Testing of Respirable Fibrous Particles

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EPA Scientific Advisory Panel Review
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I. BACKGROUND

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 that 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 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.

Using the current EPA/OPPTS health effects test guidelines for combined chronic toxicity and carcinogenicity (EPA, 1998) as a template and based on the comments and

recommendations made by a workshop expert panel on a number of scientific issues specific for fiber testing (EPA, 1996; Vu et al., 1996), EPA/OPPT developed a Proposed Guideline for combined chronic toxicity and carcinogenicity testing of fibrous particles. In July 1999, the Proposed Guideline was announced in the Federal Register for public comments (EPA, 1999). All public comments have been evaluated and a revised Draft Guideline (EPA, 2000) with some of the public comments incorporated has been prepared. This Scientific Advisory Panel (SAP) is charged to review the EPA Draft Guideline, advise on a number of remaining issues on the study protocol, and provide EPA with an opinion about the scientific validity of the test guidance.

II. MAJOR ISSUES RELATED TO FIBER TESTING

On May 8-10, 1995, a Workshop on chronic inhalation toxicity and carcinogenicity testing of respirable fibrous particles was held in Chapel Hill, North Carolina. The Workshop was sponsored by the Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency (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). The goal of the Workshop was to obtain input from the scientific community on a number of issues related to fiber testing (EPA, 1996; Vu et al., 1996). Major issues for discussion included:

- (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.

The Workshop Panel, which was composed of 19 international expert scientists in inhalation toxicology, reviewed, evaluated, and commented on the scientific issues of the Workshop. After extensive discussion and debate of the Workshop issues, the general consensus of the expert panel was:

- Chronic inhalation studies of fibers in the rat are the most appropriate tests for predicting inhalation hazard and risk of fibers to humans.
- Along with other information (decrease in body weight, systemic toxicity, etc.),

data should be obtained on lung burdens and bronchoalveolar lavage fluid (BALF) analyses in a 90-day subchronic inhalation study to assist in establishing the chronic exposure levels. In addition, BALF analysis of a number of biochemical, cellular and histopathological endpoints will help in understanding the biochemical and cellular sequence of events of particle-induced toxicity and carcinogenicity. Data obtained from lung burden analysis can also be used for the quantify aspects of risk assessment related to dosimetric adjustments before extrapolation.

- Mechanistic studies are not recommended as part of the standard chronic inhalation studies. However, there is a need for obtaining mechanistic information as far as possible during the course of subchronic or chronic inhalation studies.
- No single assay or battery of short-term assays can predict the outcome of a chronic inhalation bioassay with respect to carcinogenic effects. Several short-term *in vitro* and *in vivo* studies that may be useful to assess the relative potential of fibrous substances to cause lung toxicity/carcinogenicity were identified.

In addition, the Workshop Panel provided a number of recommendations specific for the design and conduct of chronic inhalation studies of fibers in rodents which were incorporated into the EPA Proposed Guideline for combined chronic toxicity and carcinogenicity testing of fibrous particles (EPA, 1999).

III. MAJOR ISSUES FOR THIS SAP REVIEW

Since the announcement of the Proposed Guideline (EPA, 1999) in the Federal Register on July 28, 1999, a number of comments have been received from the public on the design and conduct of chronic inhalation studies of fibers in rodents. This Scientific Advisory Panel (SAP) is charged to review the EPA Draft Guideline (which has incorporated some of the public comments) (EPA, 2000) and to provide EPA with an opinion about the scientific validity of the test guidance. Specifically, the SAP is requested to discuss and evaluate the following issues on fiber testing and to answer the questions that are related to these issues.

1. Issues on: Fiber Samples Characterization

1.1 Definition of fibers.

In the Draft Guideline, a fiber is defined as *a particle having an aspect ratio of at least 3:1 (length: diameter) and being structurally continuous. "Respirable" means that the particle in question can penetrate to the alveolar region upon inhalation. A "rat-respirable fiber" is defined as a fiber having an aerodynamic diameter of less than 3 μm* [(c) Definitions; p.2].

A public comment expressed that actual measurement of the aerodynamic diameter of fibrous materials by traditional sampling techniques is not always reliable and suggested to modify the definition of a “rat-respirable fiber” as a fiber with a geometric mean diameter (GMD) of $\leq 0.8 \mu\text{m}$.

The definition of fibers is adopted from the Workshop Panel and is widely accepted in the field. The Workshop Panel recommended that respirability should be defined on the basis of experimental data, rather than calculated data. For many types of fibers, what reaches the rat lung has been well characterized; an upper limit of $3 \mu\text{m}$ aerodynamic diameter is deemed effective in capturing rat-respirable fibers (EPA, 1996, p.8 & p.11). The Draft Guideline is defining a “rat-respirable fiber” using the aerodynamic diameter (of less than $3 \mu\text{m}$ as recommended by the Workshop Panel) also because the aerodynamic diameter but not the geometric diameter is critical for the respirability of fibrous particles; using a GMD of $\leq 0.8 \mu\text{m}$ may be too specific for defining the respirability of diverse types of fiber of various physicochemical properties, some of which (e.g., fiber density) can affect their aerodynamic diameters (and thus the respirability).

On the other hand, using a GMD of $\leq 0.8 \mu\text{m}$ to define a “rat-respirable fiber” would harmonize EPA’s guideline with fiber testing procedures and definitions being developed under the European Union (EU) Council directive 97/548/EEC. For certain fibers, a geometric diameter of $< 0.8 \mu\text{m}$ is close to an aerodynamic diameter of $< 3 \mu\text{m}$.

Question 1.1: Does the SAP agree with the definition of “rat-respirable fiber” in the Draft guideline: A “rat-respirable fiber” is defined as a fiber having an aerodynamic diameter of less than $3 \mu\text{m}$? Or, should the definition of “rat-respirable fiber” be modified as a fiber with a geometric mean diameter (GMD) of $\leq 0.8 \mu\text{m}$?

1.2 Dose/concentration.

In the “Definition” section of the Draft Guideline, it states that:
“Dose/concentration in a combined chronic toxicity and carcinogenicity study is the amount of test substance administered via the inhalation route for a period of up to 24 months. Concentration of fibrous particles is expressed as the absolute number of fibers per cubic centimeter (f/cc)” [(c) Definitions; p.1] .

One commenter suggested that a more biologically meaningful expression of concentration is the number of WHO fibers per cubic centimeter (WHO f/cc). A WHO (World Health Organization) fiber is a fiber with a diameter $< 3 \mu\text{m}$ and a length $> 5 \mu\text{m}$ and an aspect ratio (length/diameter) > 3 .

To use WHO fibers/cc in the expression of fiber concentration appears too specific. Furthermore, on selection of the test material, the Draft Guideline has specified that “rodent inhalation exposure studies should use an exposure aerosol that is, as far as

is technically feasible, enriched with the following fiber size fractions: Rat-respirable fibers with aspect ratio of at least 3:1 and aerodynamic diameter less than $3\mu\text{m}$, and human respirable fibers with lengths of at least $20\mu\text{m}$ or fibers with high aspect ratios”[(2) Control and test substances.(ii); p.4]. These fiber size selection criteria for the test materials have taken into consideration of the fibers being rat-respirable (i.e., aerodynamic diameter $< 3\mu\text{m}$) and with the greatest potential for pathogenic effects (i.e., long fibers with lengths of at least $20\mu\text{m}$) which include all WHO fibers.

Question 1.2 : Does the SAP agree with the definition of “concentration” of fibrous particles expressed in the Draft guideline ? Or, should “concentration” of fibrous particles be expressed as the number of WHO fibers per cubic centimeter of air (WHO f/cc) rather than the absolute number of fibers per cubic centimeter (f/cc) ?

It has been well demonstrated that longer fibers induce greater pathogenic effects than short fibers and that long, thin fibers (longer than $20\mu\text{m}$, thinner than $1\mu\text{m}$) have the greatest carcinogenic potency, given other parameters (e.g., dose) being equal. It is generally believed that fibers longer than $20\mu\text{m}$ are more pathogenic because they are too long to be phagocytized by alveolar macrophages and thus less likely to be cleared out of the lungs compared to short fibers.

On this basis, the Draft Guideline specifies that *“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)”*; *The fraction of long fibers ($>20\mu\text{m}$) should be specified; 10 percent to 20 percent would be appropriate”[(2) Control and test substances.(ii); p.4].*

One commenter suggested to change the length of long fibers to be enriched in the test material from $>20\mu\text{m}$ to $>15\mu\text{m}$ since the material is to be tested in the rat, and the rat alveolar macrophages are slightly smaller than human alveolar macrophages, fiber lengths somewhat smaller than $20\mu\text{m}$ may be capable of producing toxicity in the rat.

It is true that the rat alveolar macrophages are slightly smaller than human alveolar macrophages and the diameter of these cells is a limiting factor for alveolar macrophage phagocytosis; respective values for alveolar macrophage diameters have been reported to range between 10.5 and $13\mu\text{m}$ for the rat and between 14 and $21\mu\text{m}$ for humans. However, it is uncertain whether fibers (length $>15\mu\text{m}$) only slightly longer than the diameter of the rat macrophages are capable of producing toxicity in the rat since it is known that alveolar macrophages can phagocytize fibers longer than their diameter by adapting their shape.

Question 1.3 : The Draft Guideline specifies that “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)”; The fraction of long fibers (>20 μm) should be specified; 10 percent to 20 percent would be appropriate”. In view of the fact that the rat alveolar macrophages are slightly smaller than human alveolar macrophages and the material is to be tested in the rat, should the length of long fibers specified in the test material be changed from >20 μm to >15 μm ?

Another commenter pointed out that while the use of insufficient number of long fibers would produce false negative results, using too large a mass exposure would cause pulmonary overload and produce false positive results. Furthermore, at exposures approaching 200 fibers longer than 20 μm per ml of air, direct damage to the nose and eyes of the exposed animals occurs. It was suggested that as in the European Union (EU) protocol for fiber testing, limits be placed on the number of long fibers to ensure adequate test sensitivity while avoiding overload.

To maximize sensitivity of animal inhalation exposure studies to health effects of fibers, the Draft Guideline has specified the lower limit of the fraction of long fibers (>20 μm) be 10 to 20 percent”[(2) Control and test substances.(ii); p.4]. The NTP generally employs an upper limit exposure concentration of 100 mg/m³ for relatively insoluble non-fibrous particles of low toxicity. A practical upper limit of fiber concentration was discussed but was not endorsed by the Workshop Panel since it would depend on fiber type, and no one number could be determined that applies to all fibers. The Workshop Panel was aware of the potential particle “overload” using too large a mass exposure and recommended that the maximum aerosol concentration (MAC) should be based on the total number of inhaled particles (fibers and non-fibrous particles combined) and should be set based on a number of functional parameters determined in a 90-day subchronic inhalation study (EPA, 1996, p.22-23).

Question 1.4 : Does the SAP agree that a practical upper limit of fiber concentration would depend on fiber type, and no one number can be determined that applies to all fibers and, therefore, there should not be limits placed on the number of long fibers in this fiber test guideline ?

2. Issues on: Overall study Design

2.1 Animal Selection -- Species.

On this issue, the Draft Guideline specifies that: “*For the study of respirable fibrous particles via the inhalation route, the rat has been demonstrated to be the most appropriate species because of its susceptibility to fiber-induced lung diseases (fibrosis*

and lung tumors)... When the fiber in question is expected to induce mesothelioma based on analogy to potent mesothelioma inducers such as erionite and crocidolite and/or on data of short-term screening studies (e.g., intraperitoneal injection), testing in the hamster as a second rodent species is recommended since the hamster appears to be more sensitive than the rat with respect to fiber-induced mesothelioma.” [(d) Test procedure -- (1)(i); p.2].

Several public commenters stated that although the hamster is capable of maximizing the sensitivity of detecting carcinogenicity of fibers, the use of the model is controversial and the cost of such information outweighs its usefulness.

For chronic toxicity and oncogenicity studies of fibers, the Workshop Panel concluded that only the rat model has a sufficient database to be recommended for inhalation exposure studies. Although not enough was known to recommend an appropriate second species, the Workshop Panel suggested that the Agency should be encouraged to investigate health effects of fibers in hamsters and to look at the hamster as a possible second species for testing fibers since not enough is known to recommend that only one species (i.e., the rat) is needed (EPA, 1996, p.18-19).

Since the Workshop, there are new carcinogenicity data of fibers in the hamster. In addition to earlier studies showing more significant incidences of mesothelioma were induced by refractory ceramic fibers (RCF) in hamsters than in rats, a recent chronic multi-dose study with amosite asbestos has shown high incidences (4%, 26% and 20% for low-, mid-, and high-dosed groups, respectively) of mesothelioma in the hamster (McConnell et al., 1999). These data appear to further support the use of the hamster as the second species for detecting mesothelioma induction by fibers.

Question 2.1: Does the SAP agree with the Draft Guideline that for combined chronic toxicity and carcinogenicity testing of fibrous particles, the rat is the species of choice and the use of the hamster as a second rodent species is recommended (but not required) when mesothelioma is the endpoint of interest ?

2.2 Animal selection -- Sex.

The Workshop Panel concluded that presently there is no evidence of a sex difference in response to inhaled fibers, in contrast to non-fibrous particles where female rats appear to be more sensitive (thus a single sex is adequate); testing in both sexes should be encouraged (EPA, 1966; p.22). Therefore, like the current EPA/OPPTS test guidelines for combined chronic toxicity/carcinogenicity (EPA, 1998), the Draft Guideline for fibers specifies that: “*Equal numbers of animals of each sex should be used at each dose level*” [(d) Test procedure-- (1)(iii); p.2].

However, a couple of commenters expressed that for fiber testings, the use of both sexes (of animals) does not appear warranted, either in expense or the use of additional animals.

Question 2.2: Does the SAP agree that the use of both sexes of animals be required for chronic toxicity and carcinogenicity testing of fibers ? If not, which sex should be tested ?

2.3 Dose levels and dose selection.

The Draft Guideline specifies that the Maximum Aerosol Concentration (MAC) -- the highest fiber concentration to be tested in a chronic study, should be set at a level corresponding to the Maximum Tolerated Dose (MTD) at which clearance is impaired and toxicity (as determined by parameters observed during lung burden analysis and bronchoalveolar lavage fluid (BALF) analysis in a 90-day subchronic inhalation study) is observed. [(d) Test procedure --(4) (ii); p.5].

Because it is widely accepted that longer fibers induce greater pathogenic effects than short fibers and in order to maximize sensitivity of animal inhalation exposure studies to health effects of fibers, the Draft Guideline specifies that “ *An appropriate lung burden of critical fibers (long and thin) should be achieved*” for setting the MAC.

A commenter suggested that it would be more useful operationally if the word “appropriate” is defined.

For the test materials, the Draft Guideline has specified that: “*The fraction of long fibers (>20 μ m) should be specified; 10 percent to 20 percent would be appropriate*” [(2) Control and test substances -- (ii); p.4]. However, no number on the parameters in the 90-days study was suggested by the Workshop Panel since information was not sufficient to set levels for these parameters; these parameters are to be considered together in setting reasonable dose levels for the chronic study.

Question 2.3: In order to maximize sensitivity of animal inhalation exposure studies to health effects of fibers, the Draft Guideline specifies that “ *An appropriate lung burden of critical fibers (long and thin) should be achieved*” for setting the MAC. However, the word “appropriate” is not defined. Is there sufficient information to set the level of an appropriate lung burden of long fibers (>20 μ m) for defining the MAC ?

One commenter pointed out that since exposure to particles, regardless of their composition, can cause lung tumors in rats if pulmonary clearance is inhibited, the definition of MAC should be expanded to include the following statement: “*The MAC is the highest concentration of test substance that will not cause a significant impairment (retardation) of particle clearance based on assessment of clearance in a 90-day subchronic inhalation study. Significant impairment of particle clearance is a dose level that exceeds both the historical and current definition of the maximum tolerated dose (MTD)*”.

The Draft guideline specifies that “*The MAC should be set at a level corresponding to the Maximum Tolerated Dose (MTD) at which clearance is impaired and toxicity (as determined by the parameters observed during lung burden analysis and bronchoalveolar lavage fluid or BALF analysis in a 90-day subchronic inhalation study) is observed. ...*”. It does not specify what constitutes “significant impairment” of particle clearance that corresponds to that exceeding the current definition of the MTD since present information does not appear sufficient to set levels for this parameter. Furthermore, all the parameters in the 90-day subchronic study are to be considered together, rather than individually, in setting reasonable dose levels for the chronic study of fibers.

Question 2.4: Does the SAP agree that all the parameters in the 90-day subchronic study are to be considered together, rather than individually, in setting reasonable dose levels for the chronic study of fibers ? Is it necessary and, is there sufficient information to define “significant impairment” of particle clearance in a 90-day subchronic inhalation study that corresponds to that exceeding the current definition of the MTD in a chronic toxicity/carcinogenicity study ?

3. Issues on: Fiber Disposition and Dosimetry.

3.1 Bronchoalveolar lavage fluid (BALF) analysis.

On the issue of interim sacrifice, the Workshop Panel concluded that “*Interim sacrifices are essential and should be made at 3, 6, 12, 18 and 24 months in rats. The endpoints (i.e. lung burden analysis and BALF analysis) evaluated at these times should be the same as in the subchronic study. Lung burden analysis should be included in the subchronic and chronic studies even if extra animals needed to be added to the study*” [EPA, 1996, p.26 and 38].

On this basis, the Draft guideline specifies that: “*BALF analysis should be conducted at various time points (e.g., 3, 6, 12, 18 and 24 months) on subgroups of 5-6 rat/group*” [(9) BALF analysis, p.8]. “*Lung burden analyses should be conducted after 3, 6, 12, 18, and 24 months of exposure in the rat to provide data on biopersistence of the test fibers and serve as a better measure of internal dose*” [(5) Administration of the test substance. (viii), p.6]. These time points are important to follow the development/ reversibility of the lesions (BALF analysis) and the fiber disposition/ retention kinetics (lung burden analysis) so as to provide mechanistic and dosimetry data for extrapolation in risk assessment.

Two commenters expressed that analysis of BALF is useful in the 90-day studies but not needed in the chronic study and should be optional.

Question 3.1: Does the SAP agree with the Draft Guideline that bronchoalveolar lavage fluid (BALF) analysis should be evaluated at 3, 6, 12, 18 and 24

**months in the rat to follow the development/reversibility of the lesions
? Or, should the BALF analysis in the chronic study be optional ?**

3.2 Impaired lung clearance test in chronic study.

Impaired lung clearance is one of the endpoints in the 90-days subchronic study for defining the MAC. Tests for impaired clearance in the chronic inhalation study at different time points can provide information on the lung defense mechanism as a function of time to allow for the identification of any potential lung particle overload- related deviation from normal disposition and retention kinetics.

On this issue, the Workshop panel concluded that: “*At an early and late time points (e.g., 9 and 18 months of exposure), it is desirable that animals are tested for impaired clearance for a pulse of a small spherical particle*” [EPA, 1996, p.41].

On the basis of this, the Draft Guideline states that “*It is recommended that animals be tested for impaired lung clearance for a pulse of a small spherical particle at 9 and 18 months and recovery of the animals be followed with sacrifices at the same intervals as the animals exposed for 24 months*” [(10) Lung clearance, p.8].

A commenter expressed that lung clearance of particles in live animals should be measured at 9 and 18 months and recovery of the animals should be followed with sacrifices at the same intervals as the animals exposed for 24 months. Extra animals and cost will be needed if impaired lung clearance test is made mandatory in every chronic inhalation study.

Question 3.2: Does the SAP agree with the Draft Guideline that lung clearance analysis of spherical particles in animals at 9 and 18 months of exposure is recommended (but not required) in the chronic inhalation study? Or, should this be made mandatory in the chronic inhalation study ?

4. Issues on: Clinical and Histopathology Evaluation

4.1 Clinical pathology.

Consistent with the current EPA/OPPTS test guidelines on combined chronic toxicity/ carcinogenicity of chemicals [EPA, 1998], the Draft Guideline specifies that “*hematology, clinical chemistry and urinalyses should be performed from 10 animals per sex per group. The parameters should be examined at approximately 6 month intervals during the first 12 months of the study*”[(8) Clinical pathology, p.7].

One commenter expressed that chronic toxicity testing of inorganic fibers should be exempt from all clinical pathology determinations because those determinations provide little toxicological value in the assessment of fiber-induced lung diseases.

Of the larger number of natural and synthetic fibers, only a few have been tested adequately in chronic toxicity and carcinogenicity studies. There are data on translocation

for some fibers from alveolar to pleural and other sites and the toxicological effects of these fibers and their contaminants/impurities in organ systems other than the respiratory tract are largely unknown. Given the present limited knowledge of the effects of fibers in other organ systems, clinical pathology determinations in chronic toxicity and carcinogenicity of new and untested materials appear warranted.

Question 4.1: Does the SAP agree with the Draft Guideline (which is consistent with the current EPA/OPPTS test guidelines on combined chronic toxicity/carcinogenicity of chemicals) that hematology, clinical chemistry and urinalyses should be examined at approximately 6 month intervals during the first 12 months of the study ?

4.2 Ophthalmological examination.

Consistent with the current EPA/OPPTS test guidelines on combined chronic toxicity/carcinogenicity of chemicals [EPA, 1998], the Draft Guideline specifies that *“Ophthalmological examinations should be made on all animals using an ophthalmoscope or an equivalent device prior to the administration of the test substance and at termination of the study on 10 animals per sex in the high-dose and control groups. If changes in eyes are detected, all animals should be examined”*[(11) Ophthalmological examination, p.8].

One commenter stated that ophthalmological examination should be omitted since some physical effects on the eyes of animals during inhalation exposure are not generally relevant to the long-term toxicity of fibers.

Given the present limited knowledge of the effects of fibers and their contaminants/impurities in other organ systems including the eyes, ophthalmological examination in chronic toxicity and carcinogenicity of new and untested materials should be required.

Question 4.2: Does the SAP agree with the Draft Guideline (which is consistent with the current EPA/OPPTS test guidelines on combined chronic toxicity/carcinogenicity of chemicals) that ophthalmological examination should be conducted on all animals prior to the administration of the test substance and at termination of the study in the high-dose and control groups, although some physical effects on the eyes of animals during inhalation exposure are not generally relevant to the long-term toxicity of fibers ?

4.3 Histopathological evaluation.

The use of the Wagner scoring system has been suggested for the evaluation of pulmonary fibrosis to enable direct comparison of effects induced by different types of fibers. The Wagner scoring system, however, does not consider the mass of the lung tissue involved and thus has the disadvantage of being purely qualitative and inconsistently

applied. The Workshop Panel did not recommend the use of the Wagner scoring system and stated that *“To promote more quantitative evaluation, the testing guidelines should specify set procedures for grading of lesions and for lung preparation. Further research is needed before other quantitative histopathological methods can be recommended for large-scale testing. However, a promising approach could be quantitation of collagen deposits using sirius red and evaluation with polarized light.” ... “Histopathological evaluation should incorporate both qualitative description of lesions and rigorous quantitation.”* [EPA, 1996; p.32-33]

One commenter suggested that the scheduled sacrifice pathology be recorded according to the standard methods used in contemporary fiber studies, i.e., the Wagner scoring system, and that the histology slides from the scheduled sacrifices should, in addition to standard hematoxylin and eosin, be stained with Masson-Goldner's trichrome stain for collagen deposition.

Question 4.3: Before other quantitative histopathological methods can be recommended, should the Wagner scoring system and the collagen staining method be specified in the test guideline for evaluation of pulmonary lesions/fibrosis ? What other quantitative histopathological methods are currently available for grading pulmonary lesions/fibrosis that can be adopted for the inclusion in the fiber test guideline ?

Question 5.1: Does the SAP have other comments on the EPA's draft fiber test Guideline ?

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