SAP Report No. 2001-01
January 5, 2001

REPORT

FIFRA Scientific Advisory Panel Meeting, September 26, 2000, held at the Sheraton Crystal City Hotel, Arlington, Virginia

Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Session I - Test Guidelines for Chronic Inhalation Toxicity and Carcinogenicity of Fibrous Particles

NOTICE

This report has been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). This report has not been reviewed for approval by the United States Environmental Protection Agency (Agency) and, hence, the contents of this report do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

The FIFRA SAP was established under the provisions of FIFRA, as amended by the Food Quality Protection Act (FQPA) of 1996, to provide advice, information, and recommendations to the EPA Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the EPA, Office of Pesticide Programs (OPP) and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. Food Quality Protection Act Science Review Board members serve the FIFRA SAP on an ad-hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at http://www.epa.gov/scipoly/sap/ or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Larry Dorsey, SAP Executive Secretary, via e-mail at dorsey.larry@.epa.gov.

SAP Report No. 2001-01, January 5, 2001

REPORT:

FIFRA Scientific Advisory Panel Meeting, September 26, 2000, held at the Sheraton Crystal City Hotel, Arlington, Virginia

Session I - A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Test Guidelines for Chronic Inhalation Toxicity and Carcinogenicity of Fibrous Particles

Mr. Larry Dorsey
Designated Federal Official
FIFRA/Scientific Advisory Panel
Date:_____

Stephen M. Roberts, Ph.D.
Session Chair
FIFRA/Scientific Advisory Panel
Date:_____

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
September 26, 2000

Session I: Test guidelines for Chronic Inhalation Toxicity and Carcinogenicity of Fibrous Particles

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PUBLIC COMMENTS

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Written statements were received from:
none

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency regarding the review of issues pertaining to test guidelines for chronic inhalation toxicity and carcinogenicity of fibrous particles. Advance public notice of the meeting was published in the Federal Register on September 5, 2000. The review was conducted in an open Panel meeting held in Arlington, VA, on September 26, 2000. The meeting was chaired by Stephen Roberts, Ph.D. Mr. Larry Dorsey served as the Designated Federal Official.

CHARGE

1. Fiber Samples Characterization

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 μm* ? Or, should the definition of "rat-respirable fiber" be modified as a fiber with a geometric mean diameter (GMD) of $\leq$ 0.8 μm ?

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) ?

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 μ*m* to >15 μ*m* ?

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. Overall study Design

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 rodents species is recommended (but not required) when mesothelioma is the endpoint of concern ?

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 ?

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 μm) for defining the MAC ?

Question 2.4: 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. Fiber Disposition and Dosimetry

Question 3.1: Does the SAP agree with the Draft Guideline that broncho alveolar 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 ?

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. Clinical and Histopathology Evaluation

Question 4.1: Does the SAP agree with the Draft Guideline that hematology, clinical chemistry and urinalyses should be examined at approximately 6 month intervals during the first 12 months of the study ?

Question 4.2: Does the SAP agree with the Draft Guideline 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.

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: Does the SAP have other comments on the EPA's draft fiber test guideline ?

General Comments

While the Guidelines present methods for conducting a 2-year toxicity/carcinogenicity study of "fibers," they do not reflect the current "state-of-the-art" for when such studies are necessary. Part of the reason for this is that this area of toxicology is evolving rapidly and the proposed guidelines reflect the findings of a meeting held in 1995. Since 1995, much new information has been developed. In addition, long-term fiber inhalation studies require exorbitant resources, much more than for "standard" chemicals. For example, a fiber study as proposed in the guidelines using only one sex of one species would cost in excess of $2,000,000.00 and require more than 400 animals. This would be doubled if two sexes were used and quadrupled if two species were required. This is a highly impractical requirement in the context of 50-100 new fibers being introduced into the market every year. Therefore, some sort of tiered testing approach is needed before conducting these resource-intensive studies in order to reduce the number of fibers requiring testing.

The Panel noted that the document would benefit by including background information in this regard as follows:

A large body of data has recently been developed showing the critical role of biopersistence in determining the toxicity of synthetic vitreous fiber. In addition, properly conducted *in vitro* studies of dissolution properties of fibers also can provide valuable insight into the *in vivo* biopersistence of fibers (Maxim *et al.*, 1999). Biopersistence data have been shown to be predictive of the potential for toxicity and carcinogenicity. However, biopersistence data alone should not be used for classification of a new fiber; it should always include, at a minimum, some toxicity data from short-term in vivo assays. Also, it needs to be remembered that biopersistence correlation studies were only conducted for inorganic fibers; biopersistence may or may not be as important a determinant of toxicity for organic fibers. It is important that the Guidelines or a supplementary document provide a clear exposition as to the intelligible principles to be used in making decisions on the role of various approaches now available for evaluating fiber carcinogenicity and toxicity.

Other screening tests have been proposed as surrogates for long-term inhalation studies of fibers. However, the Agency needs to be cognizant of the pitfalls that arise in conducting such studies. Several authoritative groups have offered comments on the use of intra-cavitary injection of fibers to screen for toxicity and carcinogenicity (WHO, 1992; McClellan *et al.*, 1992 and NRC, 2000). These groups have either recommended against the use of such intra-cavitary injection tests or urged caution in the interpretation of the results for assessing human hazards. Nonetheless, such tests may be conducted and guidance is required on how to conduct such studies and how the results should be used in making decisions on the need for conducting chronic bioassays. These particular tests are strongly biased toward producing false-positive results because even substances that are considered highly innocuous, e.g. physiological saline, have produced tumors if the dose is high enough. Hence, when the results of such intra-cavitary injection tests show evidence that tested materials yield negative or low-potency results relative to materials previously evaluated and found negative in well conducted chronic inhalation studies

then additional chronic tests should not be required

DETAILED RESPONSES TO THE CHARGE

1. Fiber Samples Characterization

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* μ*m* ? Or, should the definition of "rat-respirable fiber" be modified as a fiber with a geometric mean diameter (GMD) of < 0.8 μm ?

The importance of characterizing airborne particles, including fibers, as to their aerodynamic size characteristics is now well established in the fields of aerosol science, industrial hygiene, and inhalation toxicology. This is the case because aerodynamic diameter is the major determinant of the deposition of airborne particles in the respiratory tract.

A definition of rat-respirable fibers as having a geometric median diameter (GMD) of less than 3.0 and geometric standard deviation of less than 3.0 would be preferable to a definition of GMD of $\leq$ 0.8 μm.

It is important to recognize that aerosols of fibers are rarely homogenous with regard to size. Thus it is important to characterize a population of fibers as to their geometric median, a measure of dispersity of size. Use of the aerodynamic diameter takes into account fibers of different densities which affect aerodynamic diameter but not the geometric mean real diameter. Organic fibers are examples of low-density particles. For example, carbon fibers will have a significantly larger diameter than a thinner glass fiber of higher density with both showing the same aerodynamic behavior. Since the aerodynamic diameter is also dependent on the aspect ratio it could be considered to express a rat-respirable fiber as having a median aerodynamic diameter of less than 3 μm with a given aspect ratio, for example 10 or 20. (See Dai and Yu, Journal of Aerosol Medicine, 11:247-258, 1998)

The Guidelines should explicitly recognize the interrelationship between (a) sampling methods, (b) evaluation of sampled material, and © the analysis and reporting of summary statistics. Investigators should be encouraged to record and report all size data collected in addition to the reporting of summary statistics such as number of WHO fibers per cubic centimeter.

The Panel recommended that the Agency consider changing the text on the selection of test material to the following (page 5, top of issue paper): "rat (instead of rodent) inhalation exposure studies should use an exposure aerosol that consists of rat-respirable fibers with aspect ratio 3:1 and aerodynamic diameter less than 3 μm. As far as is technically feasible, the aerosol should be enriched with (delete: human respirable) fibers with lengths of at least 20 μm" (delete: "or fibers with high-aspect ratios"). As currently stated in the draft guidelines, the term "human respirable" is meaningless

if these are long fibers which are not rat-respirable and therefore would not reach the lower respiratory tract of the rat.

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) ?

The Panel felt it is important for the toxicological assessment to have a good characterization of the exposure including an adequate analysis of the fiber size distribution and a determination of the absolute fiber concentration (f/cc). However, it is also important to be able to compare exposure concentrations derived from animal studies with environmental and occupational exposures so that appropriate assessments of risk can be made. Most environmental and occupational exposures are expressed in concentrations using the WHO fiber counting criteria or other similar criteria (NIOSH Method 7400).

The Panel concluded that the definition of concentration of fibrous particles should start with the absolute number of fibers/cm^3, however, it also should be emphasized that exposure concentrations should be expressed as WHO fibers /cm^3 as well as fibers greater than 20 $\mu m/cm^3$ and smaller size categories. Expressing concentrations with these different particulates and fiber categories allows an evaluation of the contribution of each category to the total aerosol. The Panel also felt that it is important to include the mass and particulate number when evaluating the aerosol.

With respect to the long fibers, the specifications in the draft guidelines should be changed such that some of the most potent human-respirable fraction - which should be enriched in the aerosol - should at the same time be rat-respirable. The sentence would read as follows (page 5, middle): "To maximize sensitivity of rat inhalation exposure studies to health effects of fibers, the test material should consist of rat-respirable fibers which (instead of "and") should be enriched with the most potent fraction of long, thin, fibers." The Agency may want to consider also identifying the length of a fiber to include 15 μm because shorter fibers may be capable of producing toxicity in the rat because of the smaller size of the rat pulmonary macrophage (~14 um) versus the human PM (~22 um). However, given the deformability of alveolar macrophages, a diameter of greater than 20 μm for the most pathogenic fibers certainly makes sense, if indeed these fibers are present under human exposure situations.

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 μ*m* to >15 μ*m* ?

The Panel suggested that the fraction of fibers >20 um should be specified and their proportion in the aerosol should be enhanced. A figure of 10-20% seems reasonable, but if it is possible to attain a higher percentage, then that should be encouraged. If it is possible to obtain fibers >20 um long, then this should be done, because there is a scientific consensus that long fibers are the ones that remain in the lung for the longest period of time and are therefore the most pathogenic fibers. However, if there are no fibers of this length, then the enrichment should be for the longest fibers occurring under human exposure conditions. The important issue is what would be expected in the air in the workplace or user environment. That mix is what should be the target mixture for the study making allowances though, for differences between human and rat respirability. The Panel was unanimous in its view that it makes little sense to create fiber lengths that could never occur in the real world exposure environment. Additionally, there should be some flexibility in selecting the number of long fibers, i.e. a requirement of 10-20% may be impractical in some situations. However, it is imperative that the reasons for not enriching the test aerosol should be clearly stated and justified.

With respect to the issue of lung overload by the long fibers, it should be remembered that any fiber longer than 20 μm will not be cleared by alveolar macrophages but will remain in the lung and mimics an "overload" condition with respect to prolonged clearance. However, this condition should not be confused with lung particle overload by non-fibrous particles which is due to a massive lung burden overwhelming alveolar macrophage mediated clearance mechanisms. Thus, given the low probability of deposition of long fibers in the rat lung, there is no danger that there is an overload occurring based on the long fibers only. However, combined deposited mass of all fibrous and non-fibrous particles in a fiber inhalation study could indeed give rise to an overload situation. This needs to be considered in the design and interpretation of such studies.

The Panel agreed that the maximum aerosol concentration (MAC) should be based on the lung burden rather than the total number of inhaled particles. The functional parameters to be used to determine the MAC from a 90-day subchronic inhalation study needs to be seen in the context of the maximum tolerated dose (MTD). The definition of MTD is difficult and somewhat different from the criteria used for determining the MTD for non-particulate materials, e.g. gases and other chemicals (McConnell, E.E., The maximum tolerated dose in particulate inhalation studies: A pathologist's point of view. Inhalation Toxicol., 8 (Suppl):111-123, 1996). From a mechanistic point of view it is a dose level that does not produce some adverse responses in the lung that would interfere with the normal physiologic processes that occur in the lung, e.g. overload, or interfere with the conduct and interpretation of the study, e.g. excessive weight loss and morbidity/mortality.

Additionally, fiber length may not be the critical determinant for a pathogenic response for some organic fibers. For example, it is difficult to generate large numbers of long/thin fibers with

nylon flock. Furthermore, cancer may not necessarily be the most relevant endpoint for some organic fibers such as nylon, which produces flockers' disease, a bronchiolar lymphocytic disease.

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?

The Panel concluded that it is difficult to set "a priori", an upper limit concentration for all possible fiber types. The purpose of the 90-day study is to establish the appropriate exposure for the 2-year study. All pulmonary responses and lung burden data should be considered in an effort to define an upper limit for a given fiber. The practical limit will depend on the type of fiber. Most of the Panel members felt that it was important to avoid overload by phagocytized fibers, because it is difficult to interpret the results of studies where overload is evident. Also, efforts should be made to limit the number of particles < 5 μm which contribute to lung burden but are not as important as long fibers in producing fiber specific responses.

One Panel member stated that because fiber dimension influences the toxicological effect, inhibition of fiber/particle clearance from the lung should not be the only determinant factor for establishing the fiber concentration for long fibers. Effects on clearance of long fibers should be considered a "pathogenic" mechanism.

2. Overall study Design:

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 rodents species is recommended (but not required) when mesothelioma is the endpoint of concern ?

The SAP noted that the Guidelines should reflect the object of a chronic toxicity/ carcinogenicity study, i.e. to determine the potential pathogenicity of a given fiber. This includes non-neoplastic as well as neoplastic disease. Rodent bioassays have proven to be fairly good predictors of carcinogenic potential in humans, at least qualitatively. However, it needs to be remembered that a rodent bioassay is a rather "blunt" instrument. While it appears to be fairly effective at identifying the carcinogenic potential of fibers, it is of less value as a measure of carcinogenic potency. Therefore, the best species for a given study, particularly of an unknown material (fiber in this case) is the one that provides the best chance to identify its non-neoplastic as well as the carcinogenic potential. This being the case, the species of choice is the one that most closely mimics humans and gives the best chance of identifying a relevant hazard. In this regard,

the rat is clearly the species of choice because it develops pulmonary fibrosis, lung neoplasms and mesothelioma in the potential target organs. In contrast, the hamster does not seem to develop lung tumors, even with highly carcinogenic fibers such as amosite asbestos (McConnell, E.E., Axten, C., Hesterberg, T.W., Chevalier, J., Miller, W., Everitt, J., Oberdorster, G., Chase, G.R., Thevenaz and Kotin, P. Studies on the inhalation toxicology of two fiberglasses and amosite asbestos in the Syrian golden hamster. Part 2. Results of chronic exposure. Inhal. Toxicol. 11:785-836, 1999). In addition, if one had relied on the hamster to establish the carcinogenic potential of chrysotile asbestos, one would have said that it was not carcinogenic, i.e. there were no lung tumors or mesotheliomas even in the presence of prominent pulmonary fibrosis (McConnell, E.E., Mast, R.W., Hesterberg, T.W., Chevalier, J., Kotin, P., Bernstein, D.M., Thevenaz, P., Glass, L.R. and Anderson, R. Chronic inhalation toxicity of a kaolin based refractory ceramic fiber (RCF) in Syrian golden hamsters. Inhalation Toxicol. 7:503-532, 1995).

The argument has been made that the hamster has a higher propensity for mesothelioma induction at a given exposure, and this has been documented with state-of-the-art inhalation studies (McConnell et al, 1995). Therefore, if one is only interested in producing the highest possible number of mesotheliomas, this species would be the one of choice. However, the rat also showed mesotheliomas with the same fibers, albeit at a lower incidence (Mast, R.W., McConnell, E.E., Hesterberg, T.W., Chevalier, J., Kotin, P., Thevenax, P., Bernstein, D.M., Glass, L.R., Miiller, W. and Anderson, R. A multiple dose chronic inhalation toxicity study of size-separated kaolin refractory ceramic fiber (RCF) in male Fischer 344 rats. Inhalation Toxicol. 7:469-502, 1995). To merely find a higher incidence of tumors from a given fiber doesn't seem to be a very resource effective study. If the rat is a better species for identifying the fibrogenic and carcinogenic potential of a given fiber (which it clearly is), then there is little to be gained from using the hamster. There is no evidence that the hamster would identify the carcinogenic potential of a given fiber that a rat would miss, but more importantly it could fail to identify the carcinogenic potential of a fiber because of its resistance to the development of lung neoplasms.

In addition, lifetime hamster studies are fraught with technical problems. Hamsters typically develop intestinal disease during the conduct of a chronic study and require therapeutic intervention to control it. This can become a serious confounder when interpreting the results. This is just as true for chemicals as well as fibers. Therefore, the use of the hamster in lieu of or in addition to the rat, is not recommended. However, if one was interested in studying the underlying mode and mechanism of action of fibers on the induction of mesothelioma, then the hamster could prove useful.

In summary, a majority of the Panel members felt that the EPA should not make the hamster study a mandatory requirement or even recommend its use in its guidelines but make it optional. The hamster could be used if mesothelioma was the only endpoint of interest because it does seem to be more sensitive than the rat for this endpoint. The importance of the hamster for this endpoint should at a minimum be mentioned in the test guidelines when there is a concern that exposure to the test material may induce mesothelioma. However, the suspicion that a fiber might cause mesothelioma should not be based solely on the result of an i.p. test, for the reasons noted above. One member noted that hamster data would be useful to avoid the argument that

certain responses are “rat specific”, but that hamsters should be recommended (not required) when mesothelioma is the endpoint of interest.

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 ?

Most members of the Panel felt that both sexes of animals should be required for the chronic toxicity and carcinogenicity testing of fibers. However, there is currently insufficient information to provide a truly informed answer to this question. There is no indication of any hormonal or immune relationship to the induction of fiber-related disease, at least for inorganic fibers. However, given that the scope of the test guidelines is to be applied to all fibrous particles (e.g., organic, inorganic, mineral) there are insufficient historical data for justifying the use of only one sex. For example, females have a greater immune response and the human response to nylon flock (an organic fiber) exhibits a strong immune component.

For mineral fibers, the robust database with various types of asbestos developed by Wagner et al., (1974) showed that males and female rats were for the most part equally sensitive to the induction of fibrosis and neoplasia with exposure to fibers. In those cases where a difference in tumor incidence was observed, a higher incidence was usually observed in males. In contrast, female rats appear to be more sensitive than males to the induction of lung tumors with non-fibrous particles as indicated in studies conducted by the NTP. However, even in those studies, both sexes showed a carcinogenic response, the incidence was just higher in females. It also needs to be remembered that the NTP studies were stopped at 2 years. If they had been allowed to proceed for most of the animal’s life, as is proposed in the EPA guidelines, the sex difference may not have been as great.

Notwithstanding this observation, it would be scientifically prudent to develop a more extensive database on the mesothelioma response in male and female rats exposed to fibers by inhalation before recommending only one sex. This would provide more comparative information.

Clearly, in those instances where fiber dissolution is significant, giving rise to the potential for systemic effects, both sexes should be used. This recommendation is based on the overwhelming evidence from chronic chemical toxicity and carcinogenicity studies of chemicals demonstrating sex specific responses (see NTP databases).

Concerning the sensitivity of the mesothelial lining, few if any studies appear to have examined whether the mesothelioma response is similar in males and females exposed to comparable levels of carcinogenic fibers by inhalation. Pott’s group (Roller et al., Environmental Health Perspectives, 105, Suppl. 5, 1997) found no sex difference in mesothelioma response in studies of over 2000 rats given i.p. injections of suspensions of fibrous or non fibrous dust, or saline. In one NTP study where mesotheliomas were induced by a chemical (i.p. injections of

acronycine) both sexes responded to a similar degree. Thus, the perception that there is not a marked sex difference in fiber-induced mesothelial cancer in male and female rats is probably correct.

Another argument for using both sexes is that, although there is little indication that there is a difference in tumor response between male and female rats after chronic fiber exposures, the use of both sexes increases the total number of animals (typically from 50 to 100) and thus sensitivity of the chronic assay.

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 μm) for defining the MAC ?

It was the consensus of the Panel that there is no universal level of long fibers that would serve as an appropriate lung burden for all types of fibers. Important differences in the biopersistence and other key physicochemical properties account for the difficulty in setting a single appropriate burden. It is also recognized that although certain fiber dimensions may be more pathogenic than others, there are important effects of the "short fiber" and non-fibrous components of the aerosol that impact the toxicity of the more pathogenic long, thin fiber component. The "appropriate" lung burden of critical fibers can only be defined through the testing of the specific fibrous particles by short-term or sub-chronic studies. Short-term or sub-chronic studies of fibrous particles should determine the biological half-life through a series of range-finding studies.

With respect to setting the MAC, the achieved lung burdens in terms of total mass as well as for fibers longer than 20 μm should be considered. This would be more appropriate than setting an aerosol concentration in terms of fibers longer than 20 μm since for different fiber types this could lead to very different lung burdens. One possibility to set an appropriate lung burden is to use data from positive, chronic rat inhalation studies with persistent fibers (e.g. amosite) and extrapolate back to a lung burden for the long fibers achieved after one or five days of exposure. This one or five day lung burden could be a goal to be achieved in the course of a chronic exposure which needs to be tested beforehand by exposing several rats for one or five days. This procedure would be preferable instead of defining a lung burden at the end of a two-year inhalation study since such lung burden depends highly on the biopersistence of a fiber. Given that the new fiber types are of a very low biopersistence, the two-year fiber burden of a biopersistent fiber will never be reached with a modern new fiber.

The suggestion that the MAC should be based on a lung burden that will not cause a significant impairment of particle clearance is too simple since that may be the case already at the

level of very low burdens of long fibers, depending on the fiber type to be tested. Impairment of clearance is only one of several parameters to be used for defining an MTD.

Question 2.4: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 ?

The Panel concluded that a weight-of-the-evidence approach should be used for establishing whether a given exposure meets the MTD. The parameters evaluated in the subchronic 90-day inhalation study should be considered together for the MTD and not one of them alone. These parameters include: lung weight; broncho alveolar lavage parameters; and lung fiber burden normalized to the exposure concentration, with special consideration of fibers. For fibers longer than 20 μm, the parameters include: quantification of cell proliferation, alveolar macrophage mediated particle clearance, histopathology, and impairment of clearance.

With respect to defining "significant impairment" of particle clearance, one could consider a doubling of the retention half time, similar to what has been proposed in the past with the term "Maximum Functionally Tolerated Dose" (MFTD). A doubling of the retention halftime is biologically significant. It should be noted that a 90-day study may not be long enough for some fibers because a steady state may not be attained within this time. The Panel noted that setting the dose will always be a problem. There is no set method for doing this. An important issue is that it will be necessary to clearly articulate the reasons for setting the dose.

3. Fiber Disposition and Dosimetry

Question 3.1:Does the SAP agree with the Draft Guideline that broncho alveolar 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?

Most members of the Panel felt that BALF collection should be mandatory only if the EPA believes the data derived from this analysis is necessary to properly interpret the study results. There was a general consensus among Panel members that BALF was particularly useful in the pre-chronic (dose-setting) phase of the study. However, several members questioned its value in a chronic study. The object of a chronic study is primarily to identify the chronic toxicity and pathogenicity (especially fibrogenic and carcinogenic potential) of a given fiber. It was not clear how BALF data would aid in determining these endpoints, which rely totally on the histopathologic observations. Also, it was noted that toward the end of the study (after 18 months), the normal aging changes such as spontaneous neoplasia, etc. could confound

interpretations. Other members of the Panel felt that BALF analysis evaluation at interim time points (3,6,12,18 & 24 months) would be very useful for an overall evaluation of the fiber toxicity. However, a decision to include all time points should be left to the individual study design, and the interim time points could be omitted providing it is done at the end of this study (24 months) and at the midpoint (12 months). BALF analysis can be of value for:

-Quantification of chronic inflammation, even in the absence of overt fibrosis or lung neoplasms. This quantification would be a valuable addition to the semi-quantitative subjective description by histopathology as mild, moderate, severe etc.
-Obtaining most complete information on events in the target tissue of greatest concern, i.e. the lower respiratory tract.
-Using resources most effectively: The additional costs involved are minor compared to the overall cost of the study.
-Use as a bookmarker for the cleanliness of the testing conditions during the exposure.

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 ?

A major concern in the conduct of chronic carcinogenicity and toxicity studies is the potential for setting the highest dose (exposure) levels at a level that exceeds the Maximum Tolerated Dose resulting in non-specific false-positive effects. This is an especially critical issue for inhalation studies in which there is a build-up of inhaled material in the lungs as the exposures are continued and evidence of the non-specific pathology may not be evident until after several months of exposure. The non-specific effects are manifest as inflammation, fibrosis, impaired clearance and, ultimately, the development of lung tumors in the rat. This has frequently been referred to as the "overload phenomena" (McClellan, 1996; Wolff *et al.*, 1987). The effect is not always readily apparent from the results of the 90-day study.

A National Toxicology Program advisory committee addressed the issue of setting exposure levels for chronic inhalation studies, albeit, they were addressing non-fibrous particulates (Lewis *et al.*, 1989). That committee recommended the use of lung clearance studies with test aerosol as one approach to evaluating whether "overload" had occurred. The Committee also noted the specialized nature of this test procedure and the extent to which the capability might not be available in all laboratories.

However, the Panel felt that the use of the aerosol clearance tests at 9 and 18 months should be optional. While it can certainly provide valuable information to complement other data in evaluating the appropriateness of the exposure levels used, it is important to recognize decisions concerning the appropriateness of exposure levels should be made on a weight-of-the-

evidence basis. This includes consideration of the pathology present and, most importantly, information on lung burdens of the material being evaluated. Information on whether lung burdens are proportional or not proportional to exposure concentration over the range of exposure levels is especially useful and does not require detailed assessment of clearance (Wolff *et al.*, 1987). In that case, the result could be equivalent to test particle clearance of spherical particles.

The Panel also noted that special equipment is required for performing lung clearance analysis with inhaled test particles and requires additional time and costs and, most importantly, specific expertise. For example, it is becoming harder to obtain and work with radio-labeled particles in general, yet other methods will be invasive and more costly.

Finally, the Panel was concerned with how the results of such studies would be used in the risk assessment process. For example, how would the Agency treat the results where there is impaired clearance or an increase PMNs in BALF. What is the definition of "impaired" or "increased"? Does either mean that the study is not valid? Would a tumor response at a dose level where clearance was impaired be ignored? The Panel feels that these issues need to be carefully considered before making clearance studies mandatory.

4. Clinical and Histopathology Evaluation

Question 4.1:Does the SAP agree with the Draft Guideline that hematology, clinical chemistry and urinalyses should be examined at approximately 6 month intervals during the first 12 months of the study ?

The Panel concluded that there was no evidence that these assays provided useful information in fiber inhalation studies with rodent models. Therefore, there was a consensus that these tests are not needed unless the fiber under test was known to have special properties of “chemical toxicity”. They are best incorporated into the pre-chronic testing schemes since there are many compromising factors that limit the utility of clinical pathology in long-term bioassays. In the absence of demonstrable histopathology or body weight effects, general clinical chemistry and urinalysis measures during pre-chronic studies used to select doses for chronic studies are more useful for determining a NOAEL than for establishing toxicity sufficient for establishing a MAC.

If the Agency intends to use these assays, the Panel encourages consideration of efforts at international harmonization in this regard (Weingand et al. Harmonization of animal clinical pathology testing in toxicity and safety studies. *Fund. Appl. Toxicol.*, 29(2): 198-201, 1996).

Question 4.2:Does the SAP agree with the Draft Guideline 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.

Most Panel members felt that ophthalmological exams have limited usefulness. There is no evidence that such effects (except locally from airborne fibers depositing externally) should be expected. Also, there is a question of cost/benefit. It was suggested that they be mandatory only when there is reason to do so from human experience with the particular fiber. While some synthetic inorganic and mineral fibers may pose a minor risk to the eyes due to physical irritation, it is unknown what effects may be caused from exposure to organic fibers because of their chemical composition. However, these examinations have little merit if the chronic inhalation study is conducted by nose-only administration.

One Panel member felt that the Guidelines should at least encourage the use of ophthalmological examinations. He noted that while evidence of ophthalmological effects are not directly relevant to assessing pulmonary toxicity, the documentation of the presence or absence of ophthalmological effects can be useful in evaluating occupational exposure limits in the absence of specific human observations to the contrary. If the Agency requires or encourages their use, there should be clear guidance as to what is meant by an ophthalmological examination. Does this entail a simple view of the external eye or a more sophisticated technique such as a slit lamp examination?

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 ?

The Panel noted that the Wagner scoring system has proved useful for providing a consistent and systematic reference for parenchymal disease. It also can be of value when attempting to compare the pathogenic effects of one fiber to another. However, it has the problem of not being able to determine the magnitude or distribution of early fibrosis. This deficiency can be remedied to some degree if a consistent approach is taken to recording subsets of the pathology examination. For example, the pathologist needs to record and grade his/her findings for airway disease, nature of inflammatory component, effects at the TB junction, more distal areas of the lung, and pleura. There also was a suggestion that “image analysis” would provide an opportunity to quantify the intensity and distribution of the fiber exposure in the lung. The use of “image analysis” also provides the capability to assess severity.

Replicate sections of lung should be stained with an appropriate stain for identifying collagen. However, there are many stains for collagen. There is no need to dictate a single stain as is done in the Guidelines.

Question 5: Does the SAP have other comments on the EPA's draft fiber test Guidelines.

The Panel had several additional comments that would enhance the Guidelines. They are noted below, but not necessarily in order of importance:

- The preamble to the Guidelines would benefit from a statement that clearly emphasizes that the major purpose of these guidelines is to provide data for quantitative risk-assessment as EPA is required to perform.

- In the preamble it would be helpful to indicate the value of a tiered testing system of which the chronic 2-year bioassay is only the last phase. At the same time, it would be useful to also attempt some harmonization with respect to guidelines that have been and are being developed within the European Union, particularly the new guideline on sub-chronic fiber testing. A point that is missing in the European guidelines and which should be included in the EPA Guidelines is the focus on the lung fiber burden as a dose-metric, rather than the focus on the aerosol alone.

- It would be useful to point out in the section of the Guidelines dealing with aerosol characterization that there is a need to clean up the fiber aerosol from non-fibrous particles as much as possible. Also, there remains the concern that not all "human respirable" fibers can be tested in animal models. EPA should consider addressing this concern through possible collaborations with other agencies.

- The definition given on page 1 of the guidelines concerns concentration and not dose. These two terms should be strictly kept separate. The dose, in contrast, would be the dose deposited upon inhalation or the dose retained in the lung after a certain time post-exposure.

- Page 2, a NOEL is listed here, yet the text relates to a NOAEL.

- On page 6, it is specified here that the lung burden and fiber size distribution should be reported as "number of fibers per gram of dry lung tissue". The number of fibers in the lung should be expressed in multiple ways. Number of fibers/lung, number of fibers/mg dry lung and fibers/g wet lung. Incorporating these data makes it easier to compare one study to another, given also that the dry lung weight vs. wet lung weight ratio may be different in the lung when fibrosis or tumors are present. Fiber numbers should also be expressed by fiber length, e.g. WHO fibers, f >10, f >15, f >20 μm in length. In addition to the time points noted in the Guidelines, fiber burdens should be evaluated at the terminal sacrifice.

- On page 7, paragraph (ii), a number of detailed clinical observations are described, including autonomic effects and central nervous system effects which appear to require major testing. These detailed tests are not necessary for a routine examination. The Guidelines need to be clearly written in this regard to preclude confusion on the part of the user.

- The Agency should give serious consideration to adding recovery groups as part of the guidelines. These groups of animals (typically 6-10/dose group) can provide insight into the progression or regression of a given lesion in the absence of further exposure. In addition, they are of great value in evaluating whether a given fiber persists in the lung for long periods of time.

- The number of animals in the study does not compare to what is noted in on page 3, paragraph (iv) and will need to be recalculated. The panel agrees that there should be a core group of 50 animals/sex/dose for evaluating the carcinogenic potential of a fiber.

- Page 12, paragraph (iii). The rib cage and diaphragm should also be required for histopathological examination. The section of diaphragm for histology should be cut in a manner to show the muscular as well as the non-muscular portion.

- Page 12, paragraph (iv). Karnovsky's fixative is only necessary if one is interested in conducting electron microscopic studies. The choice of fixative should be optional.

- The Guidelines should explicitly note that Nose-Only Exposures may be conducted as an alternative to Chamber Exposures. In recent years nose-only exposures have been the exposure technique of choice for fiber studies, although whole-body exposure may be applicable if it can be documented that sufficient fibers reach the gas-exchange region of the lung.

- The Guidelines in several places make note of the use of material enriched with regard to respirable fibers. There may be situations when it is appropriate to enrich the test material with regard to the respirable fraction. However, it must be kept in mind that enrichment procedures create an artificial mix of test material that in turn may result in a test atmosphere that is quite different than atmospheres encountered during production or use of this fiber product. The goal of test procedures should be to develop information relevant to evaluating human hazards/risks under "real-world" exposure conditions. To help in understanding how an "enriched" test material relates to materials encountered in production or use of a fiber product, it is important that detailed information be available on the enrichment process, including the extent to which respirable fibers are increased in concentration during the process. The extent to which the enrichment process may have altered fiber dimensions should also be documented. Industrial hygiene information on occupational exposures should play an important role in deciding when it is necessary to test materials in chronic bioassays and to select appropriate exposure levels.

- The document needs to have a clear statement that while the Guidelines will have application to "testing" organic fibers, that they were derived from experience with inorganic fibers and that additional considerations may be necessary for study of organic fibers.

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