

US EPA ARCHIVE DOCUMENT

OPPTS 870.8355 Combined Chronic Toxicity/Carcinogenicity Testing of Respirable Fibrous Particles.

(a) **Scope -- (1) Applicability.** This guideline is intended to meet testing requirements of the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this OPPTS test guideline are 40 CFR 798.3320 Combined Chronic Toxicity/Oncogenicity, and EPA-748-R-96-001 Workshop Report on Chronic Inhalation Toxicity and Carcinogenicity Testing of Respirable Fibrous Particles.

(b) **Purpose.** The objective of this combined chronic toxicity/carcinogenicity study is to determine the effects of a fibrous substance in at least one mammalian species following prolonged and repeated inhalation exposure. The application of this guideline should generate data which identify the majority of chronic and carcinogenicity effects and determine dose-response relationships. The design and conduct should allow for the detection of neoplastic and non-neoplastic effects of the target tissues as well as a determination of the potential of a fibrous substance and/or its contaminants/impurities to induce general toxicity, including neurological, physiological, biochemical, and hematological effects and exposure-related morphological (pathology) effects.

(c) **Definitions.** The definitions in section 3 of TSCA and the definitions in 40 CFR Part 792--Good Laboratory Practice Standards (GLP) apply to this guideline. The following definitions also apply to this guideline.

Carcinogenicity is the development of neoplastic lesions as a result of the repeated daily exposure of experimental animals to the test substance by the inhalation route of exposure.

Chronic toxicity is the adverse effects occurring as a result of the repeated daily exposure of experimental animals to the test substance by the inhalation route of exposure.

Cumulative toxicity is the adverse effects of repeated dose occurring as a result of prolonged action on, or increased concentration of, the administered test substance in susceptible tissues.

Dose/concentration in a combined chronic toxicity/carcinogenicity study is the amount of test substance administered via inhalation routes for a period of up to 24 months. Concentration of fibrous particles is expressed as absolute number of fibers per cubic centimeter (f/cc). Gravimetric concentration expressed as milligrams per cubic meter

(mg/m³) is used for daily monitoring of the generated aerosols in order to achieve the intended number of fiber per unit of aerosol volume (f/cc).

Fibrous particles/fibers are generally defined as elongated particles with a length-to-diameter ratio (i.e., aspect ratio) equal to or greater than 3 to 1. This definition is presumed to include particles with varying shapes such as rod-like, curly, or acicular (needle-like) shapes, and having different structural units commonly referred to as fibers, fibrils, or whiskers.

No-observed-effect-level (NOEL) is the maximum dose used in a study which produces no observed adverse effects.

Respirable means that the particle in question can penetrate to the alveolar region upon inhalation. There are considerable differences in fiber respirability between laboratory rodents and humans. A "rat-respirable fiber" is defined as a fiber having an aerodynamic diameter of less than 3 μm . A fiber having an aerodynamic diameter of less than 5 μm is respirable by humans. (Aerodynamic diameter, the most important determinant of the respirability of a fiber, is different from its actual, geometric diameter. The aerodynamic diameter of a fiber is dependent on its density and aspect ratio. For example, fibers having actual diameters of 0.25 to 2.0 μm would have aerodynamic diameters three to four times their actual diameters if their density is 1g/cm³ and their lengths are between 10 and 150 μm).

Target organ is any organ of a test animal showing evidence of an effect induced by a test substance.

(d) **Test procedure** -- (1) **Animal selection** -- (i) **Species and strain.** 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). Commonly used laboratory strains should be employed. The strain selected should be susceptible to the carcinogenic or toxic effect of fibrous particles. The criteria for a suitable strain include:

- (A) A low background rate of neoplasia.
- (B) A low background rate of pulmonary disease.
- (C) Longevity.
- (D) A history of laboratory use.

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. If other species are used, the tester should

provide justification/reasoning for the selection.

(ii) **Age/weight.** (A) Testing should be started with young healthy animals as soon as possible after weaning and acclimatization.

(B) Dosing should generally begin no later than 8 weeks of age.

(C) At commencement of the study, the weight variation of animals used should not exceed 20 percent of the mean weight for each sex.

(iii) **Sex.** (A) Equal numbers of animals of each sex should be used at each dose level.

(B) Females should be nulliparous and nonpregnant.

(iv) **Numbers.** (A) At least 100 rodents (50 males and 50 females) should be used at each dose level and concurrent control group. At least 40 additional rodents (20 males and 20 females) should be used for satellite dose groups and the satellite control group. The purpose of the satellite groups are for interim sacrifices for lung burden analysis, BALF (bronchoalveolar lavage fluid) analysis and the evaluation of pathology other than neoplasms (e.g., fibrosis).

(B) For a meaningful and valid statistical evaluation of long term exposure and for a valid interpretation of negative results, the number of animals in any group should not fall below 50 percent at 18 months for rats and 15 months for hamsters. Survival in any group should not fall below 25 percent at 24 months for rats and 18 months for hamsters.

(C) To avoid bias, the use of adequate randomization procedures for the proper allocation of animals to test and control groups is required.

(D) Each animal should be assigned a unique identification number. Dead animals (and their preserved organs) and tissues, and microscopic slides should be identified by reference to the unique numbers assigned.

(v) **Husbandry.** (A) Animals should be housed individually during exposure in inhalation studies.

(B) The temperature of the experimental animal rooms should be at $22 \pm 3^{\circ}\text{C}$.

(C) The relative humidity of the experimental animal rooms should be 50 ± 20 percent.

(D) The daily light cycle should be maintained at 12 h light and 12 h dark, whether

artificial or natural.

(E) Control and test animals should be fed from the same batch and lot. The feed should be analyzed to assure uniform distribution and adequacy of nutritional requirements of the species tested and for impurities that might influence the outcome of the test. Animals should be fed and watered ad libitum with food replaced at least weekly.

(F) The study should not be initiated until animals have been allowed a period of acclimatization/quarantine to environmental conditions, nor should animals from outside sources be placed on test without an adequate period of quarantine.

(2) **Control and test substances.** (i) One lot of the test substance should be used throughout the duration of the study if possible, and the research sample should be stored under conditions that maintain its purity and stability. Prior to the initiation of the study, there should be a characterization of the test substance, including the purity and physicochemical properties (e.g., fiber morphology, dimension, size distribution, aerodynamic diameter, chemistry, density, dissolution rate (K_{dis}), surface characteristics, the ability of a fiber to split longitudinally or cross-sectionally) of the test fiber and, if possible, the name and quantities of contaminants and impurities.

(ii) 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); therefore, 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 3mm, and human respirable fibers with lengths of at least 20 μm or fibers with high aspect ratios. The fraction of long fibers ($>20 \mu\text{m}$) should be specified; 10 percent to 20 percent would be appropriate. The aerosolized fibers should be discharged to Boltzmann equilibrium before being delivered to the test species.

(3) **Control groups.** A concurrent control group (50 males and 50 females) and a satellite control group (10 males and 10 females) are required. These groups should be untreated. Animals in the satellite control group should be sacrificed at the same time the satellite test group is terminated. A positive control group may not need to be included in every study, but each new test system (including use of a different animal species and strain) should be validated with a positive control material.

(4) **Dose levels and dose selection.** (i) For risk assessment purposes, at least three dose levels should be used, in addition to the concurrent control group. Dose levels should be spaced to produce a gradation of effects. A rationale for the doses selected must be

provided.

(ii) The highest fiber concentration to be tested in a chronic study is known as the maximum aerosol concentration or MAC. The MAC should be based on the total number of inhaled particles (fibers and non-fibrous particles combined). The MAC should be considered based on a combination of the following parameters determined during lung burden analysis and BALF (bronchoalveolar lavage fluid) analysis in a 90-day subchronic inhalation study: altered alveolar macrophage mediated particle clearance rate, fiber lung burden normalized to exposure concentration, cell proliferation, histopathology, inflammation (marker enzyme activities, total protein content, total cell and differential counts, e.g., quantitatively determined as percentage increase in polymorphonuclear leukocytes [PMNs], and cell viability in lung lavage samples) and lung weight. An appropriate lung burden of critical fibers (long and thin) should be achieved. In addition, impairment of clearance should be assessed in a 90-day subchronic inhalation study via challenge with a tagged particle. Clearance should be assessed after the 90-day exposure period and the clearance of the labeled particles should be measured over a recovery period for another 3 months. 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 listed above) is observed. All the parameters in the 90-day study should be considered together, rather than individually, in an attempt to define a MAC, and should be presented to the agency for evaluation before the chronic study is conducted.

(iii) The intermediate dose levels should be spaced to produce a gradation of toxic effects.

(iv) The lowest dose level should produce minimal or no evidence of toxicity.

(5) **Administration of the test substance.** Inhalation is the major route of human exposure of fibrous particles, and chronic inhalation studies in rodents are deemed appropriate tests for evaluating inhalation hazard and risk of fibers to humans. Either nose-only or whole-body exposure can be used.

(i) The animals should be exposed to the test substance, for 6 h/day on a 7-day per week basis, for a period of at least 24 months in rats and 18 months in hamsters. However, based primarily on practical considerations, exposure for 6 h/day on a 5-day per week basis is acceptable.

(ii) The animals should be tested in dynamic inhalation equipment designed to sustain a minimum air flow of 10 air changes per hour, an adequate oxygen content of at least 19 percent, and uniform conditions throughout the exposure chamber. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into

surrounding areas.

(iii) The selection of a dynamic inhalation chamber should be appropriate for the test substance and test system. Where a whole body chamber is used, individual housing must be used to minimize crowding of the test animals and maximize their exposure to the test substance. To ensure stability of a chamber atmosphere, the total volume occupied by the test animals should not exceed 5 percent of the volume of the test chamber. The animals should be acclimated and heat stress minimized.

(iv) The temperature at which the test is performed should be maintained at $22 \pm 3^{\circ}\text{C}$. The relative humidity should be maintained between 50 ± 20 percent.

(v) The rate of air flow should be monitored continuously but recorded at least every 30 minutes.

(vi) Temperature and humidity should be monitored continuously but should be recorded at least every 30 minutes.

(vii) During the development of the generating system, fiber/particle size analysis should be performed to establish the stability of aerosol concentrations with respect to fiber size. During exposure, analysis should be conducted to determine the consistency of fiber size distribution and the actual concentrations of the test substance. The frequency of exposure atmosphere monitoring should be daily for mass concentration, weekly for fiber concentration and bivariate size distribution.

(viii) The actual concentrations of the test substance should be measured in the breathing zone. 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. Data also should be obtained on fiber deposition in the nasal cavity and the fiber burden in the thoracic lymph nodes. The fibers should be analyzed for number, bivariate size distribution and chemistry. For fiber burden analysis, one of the two lungs (left or right) should be used, rather than only the accessory lobe. Five to six animals per exposure group should be studied at each time point. Lung burden and fiber size distribution should be reported as number of fibers per gram of dry lung tissue. The burden should be extrapolated to the whole lung. The method for lung burden analysis ("lung digestion") must be validated. It is recommended to include "recovery" groups of animals from exposure at 3, 6, 12, and 18 months and then hold until 24 months for evaluation.

(ix) Feed should be withheld during exposure. Water may also be withheld during exposure.

(6) **Observation period.** The chronic inhalation exposure study with fibers should be a lifetime study. The animals should be observed for their life span after the exposure duration is completed (at least 24 months for rats and 18 months for hamsters); final sacrifice should be carried out only when survival of the control group reaches 20 percent.

(7) **Observation of animals.** (i) Observations should be at least twice each day for morbidity and mortality. Appropriate actions should be taken to minimize loss of animals from the study (e.g., necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals). General clinical observations should be made at least once a day.

(ii) A careful clinical examination should be made at least once weekly. Observations should be detailed and carefully recorded, preferably using explicitly defined scales. Observations should include, but not be limited to, evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of motor activity, gait and posture, reactivity to handling or sensory stimuli, grip strength and stereotypies or bizarre behavior (e.g., self-mutilation, walking backwards).

(iii) Body weights should be recorded individually for all animals: Once a week during the first 13 weeks of the study and at least once every 4 weeks thereafter unless signs of clinical toxicity suggest more frequent weighing to facilitate monitoring of health status.

(iv) Moribund animals should be removed and sacrificed when noticed and the time of death should be recorded as precisely as possible. At the end of the study period, all survivors should be sacrificed.

(8) **Clinical pathology.** 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. If possible, these collections should be from the same animals at each interval. If hematological and biochemical effects are seen in the subchronic study, testing should also be performed at 3 months. Overnight fasting of animals prior to blood sampling is recommended.

(i) Hematology. The recommended parameters are: Hemoglobin and hematocrit concentrations, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, white blood cell count, differential leukocyte count, platelet count and a measure of clotting potential, such as prothrombin time or thromboplastin time.

(ii) Clinical chemistry. (A) Parameters which are considered appropriate to all studies are electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance and signs of clinical toxicity.

(B) The recommended clinical chemistry determinations are potassium, sodium, glucose, total cholesterol, urea nitrogen, creatinine, total protein, and albumin. More than two hepatic enzymes (such as alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, or gamma glutamyl transferase.) should also be measured.

(iii) Urinalyses. The following determinations should be made from either individual animals or on a pooled sample per sex per group: Appearance (volume and specific gravity), pH, protein, glucose, ketones, bilirubin, occult blood (semiquantitatively), and microscopy of sediment (semiquantitatively).

(9) **Bronchoalveolar lavage fluid (BALF) analysis.** BALF analysis should be conducted at various time points (e.g., at 3, 6, 12, 18 and 24 month) on subgroups of 5-6 rats/group. Lavage parameters to be determined include: total cell count, differential cell counts (PMN, alveolar macrophages, lymphocytes, and others), total protein, LDH and beta-glucuronidase as examples of cytoplasmic and lysosomal enzymes. It is recommended to include "recovery" groups of animals from exposure at 3, 6, 12, and 18 months and then hold until 24 months for evaluation.

(10) **Lung clearance.** 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.

(11) **Ophthalmological examination.** 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.

(12) **Gross necropsy.**(i) A complete gross examination should be performed on all animals, including those which died during the experiment or were killed in a moribund condition.

(ii) The liver, lungs, kidneys, brain, spleen, and gonads should be trimmed and weighed wet, as soon as possible after dissection to avoid drying. The organs should be weighed from interim sacrifice animals as well as from at least 10 animals per sex per group at

terminal sacrifice.

(iii) In inhalation studies of fibers, the entire respiratory tract, including nose, pharynx, larynx, paranasal sinuses, lungs, trachea and pleura should be examined and preserved.

(iv) Inflation of lungs with a fixative is the optimal method for preservation of these tissues. The proper inflation and fixation of the lungs in inhalation studies is essential for appropriate and valid histopathological examination. To allow the grading of lesions and better evaluation of subtle changes, the lungs from the scheduled sacrifices are recommended to be inflated with a fixative (e.g., Karnovsky's fixative) via the trachea to a constant pressure of 30 cm. water for at least two hours.

(v) The following organs and tissues, or representative samples thereof, should be preserved in a suitable medium for possible future histopathological examination:

(A) Digestive system – salivary glands, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, gallbladder (when present).

(B) Nervous system – brain (multiple sections), pituitary, peripheral nerves, spinal cord (three levels), eyes (retina, optic nerve).

(C) Glandular system – adrenals, parathyroids, thyroid.

(D) Cardiovascular/hematopoietic system – aorta (thoracic), heart, bone marrow, lymph nodes, spleen, thymus.

(E) Urogenital system – kidneys, urinary bladder, prostate, testes/epididymides, seminal vesicles, uterus, ovaries, female mammary gland.

(F) Other – all gross lesions and masses, skin.

(vi) Information from clinical pathology and other in-life data should be considered before microscopic examination, since these data may provide significant guidance to the pathologist.

(13) **Histopathology.** (i) The following histopathology should be performed:

(A) Target organs/tissues in all animals. Organs/tissues of the respiratory tract represent the target organs/tissues for evaluating effects of inhaled fibers. Major effects include pulmonary fibrosis, lung tumors and mesotheliomas. Special attention to examination of the lungs of rodents should be made for evidence of infection since this provides an

assessment of the state of health of the animals.

(B) Full histopathology on the organs and tissues, listed under paragraph (d)(12)(v) of this guideline of all animals in the control and high dose groups and of all animals that died or were killed during the study.

(C) All gross lesions in all animals.

(ii) If the results show substantial alteration of the animal's normal life span, the induction of effects that might affect a neoplastic response, or other effects that might compromise the significance of the data, the next lower levels should be examined fully as described in paragraph (d)(12)(i) of this guideline.

(iii) An attempt should be made to correlate gross observations with microscopic findings.

(iv) Tissues and organs designated for microscopic examination should be fixed in 10 percent buffered formalin or a recognized suitable fixative as soon as necropsy is performed and no less than 48 hours prior to trimming. Tissues should be trimmed to a maximum thickness of 0.4 cm for processing.

(v) Histopathological evaluation should incorporate both qualitative description of lesions and rigorous quantitation.

(e) **Data and reporting -- (1) Treatment of results.** (i) Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions, the types of lesions and the percentage of animals displaying each type of lesion.

(ii) All observed results, (quantitative and qualitative) should be evaluated by an appropriate statistical method. Any generally accepted statistical methods may be used; the statistical methods including significance criteria should be selected during the design of the study.

(2) **Evaluation of study results.** (i) The findings of a combined chronic toxicity/carcinogenicity study should be evaluated in conjunction with the findings of previous studies and considered in terms of the toxic effects, the necropsy and histopathological findings. The evaluation will include the relationship between the dose of the test substance and the presence, incidence and severity of abnormalities (including behavioral and clinical abnormalities), gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects.

(ii) Non-neoplastic and neoplastic endpoints recorded should include, but not be limited to, epithelial hyperplasia, alveolar bronchiolization, metaplasia, adenomas, mesotheliomas, and carcinomas. A dissecting microscope should be used to examine for mesotheliomas. In distinguishing between hyperplasia and mesothelioma, standard diagnostic criteria should be applied to identified lesions. Established published guidelines on the use of blinding in histopathology should be followed, e.g., those published by the Society of American Pathologists.

(iii) In order for a negative test to be acceptable, it should meet the following criteria-- no more than 10 percent of any group is lost due to autolysis, cannibalism, or management problems, and survival in each group is no less than 50 percent at 15 months for hamsters and 18 months for rats. Survival should not fall below 25 percent at 18 months for hamsters and 24 months for rats. For acceptance of the results of a chronic inhalation exposure study with fibers as negative, the study must have been designed and conducted according to the criteria outlined previously, the health effects of concern must not be significantly more frequent in the exposure groups than in the control group. Sample sizes for toxicity studies should be large enough to detect a specific effect level (e.g., a 10% change in fibrosis or tumor) with $\alpha = P$ (Type I error) = 0.05 and power = $1 - P$ (Type II error) ≥ 0.80 .

(iv) The use of historical control data from an appropriate time period from the same testing laboratory (i.e., the incidence of tumors and other suspect lesions normally occurring under the same laboratory conditions and in the same strain of animals employed in the test) is helpful for assessing the significance of changes observed in the current study.

(3) **Test report.** (i) In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, 40 CFR part 160, the following specific information should be reported:

(A) Test substance characterization should include:

(1) Chemical identification.

(2) Lot or batch number.

(3) Physicochemical properties (i.e., fiber morphology, dimension, size distribution, aerodynamic diameter, chemistry, density, solubility, surface characteristics, the ability of a fiber to split longitudinally or cross-sectionally).

(4) Purity/impurities.

(B) Test system should contain data on:

(1) Species and strain of animals used and rationale for selection if other than that recommended.

(2) Age including body weight data and sex.

(3) Test environment including cage conditions, ambient temperature, humidity, and light/dark periods.

(C) Test procedure should include the following data:

(1) Method of randomization used.

(2) Full description of experimental design and procedure.

(3) Dose regimen including levels, methods, and volume.

(D) Test conditions. The following exposure conditions must be reported.

(1) Description of exposure apparatus including design, type, dimensions, source of air, system for generating particulates and aerosols, method of conditioning air, treatment of exhaust air and the method of housing the animals in a test chamber.

(2) The equipment for measuring temperature, humidity, and fiber/particulate aerosol concentrations and size should be described.

(E) Exposure data. These should be tabulated and presented with mean values and a measure of variability (e.g. standard deviation) and should include:

(1) Airflow rates through the inhalation equipment.

(2) Temperature and humidity of air.

(3) Actual (analytical or gravimetric) concentration in the breathing zone.

(4) Nominal concentration (total amount of test substance fed into the inhalation equipment divided by volume of air).

(5) Fiber and particle size distribution, and calculated mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD).

(6) Explanation as to why the desired chamber concentration and/or fiber size could not be achieved (if applicable) and the efforts taken to comply with this aspect of the guidelines.

(F) **Test results** (1) Group animal data. Tabulation of toxic response data by species, strain, sex and exposure level for:

(A) Number of animals exposed.

(B) Number of animals showing signs of toxicity.

(C) Number of animals dying.

(2) Individual animal data. Data should be presented as summary (group mean) as well as for individual animals.

(A) Time of death during the study or whether animals survived to termination.

(B) Time of observation of each abnormal sign and its subsequent course.

(C) Body weight data.

(D) Feed and water consumption data, when collected.

(E) Results of ophthalmological examination, when performed.

(F) Results of hematological tests performed.

(G) Results of clinical chemistry tests performed.

(H) Results of urinalysis tests performed.

(I) Results of lung burden analysis.

(J) Results of BALF (bronchoalveolar lavage fluid) analysis.

(K) Necropsy findings including absolute/relative organ weight data.

(L) Detailed description of all histopathological findings. Histopathological evaluation should incorporate both qualitative description of lesions and rigorous quantitation.

(M) Statistical treatment of results where appropriate.

(N) Historical control data.

(f) **Quality assurance.** A system should be developed and maintained to assure and document adequate performance of laboratory staff and equipment. The study must be conducted in compliance with the GLP regulations as described by the Agency (40 CFR parts 160 and 792) and the OECD Principles of GLP (ISBN 92-64-12367-9).

(g) **References.** The following references should be consulted for additional background information on this guideline.

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(13) United States Environmental Protection Agency. Health Effects Test Guidelines_Combined Chronic Toxicity/Oncogenicity. 40 CFR Part 798.3320 Federal Register. pp. 165-172, July 1, 1998.

(14) United States Environmental Protection Agency. Office of Pollution Prevention and Toxics. Workshop on Chronic Inhalation Toxicity and Carcinogenicity Testing of Respirable Fibrous Particles. EPA-748-R-96-001, January 1996.

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(16) World Health Organization (WHO). Part I. Environmental Health Criteria 6, Principles and Methods for Evaluating the Toxicity of Chemicals. WHO, Geneva. (1978).