

§ 799.9420

40 CFR Ch. I (7-1-07 Edition)

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(12) Weingand K., Brown G., Hall R. et al. (1996). Harmonization of Animal Clinical Pathology Testing in Toxicity and Safety Studies. *Fundam. and Appl. Toxicol.* 29:198-201.

[65 FR 78797, Dec. 15, 2000]

§ 799.9420 TSCA carcinogenicity.

(a) *Scope*. This section is intended to meet the testing requirements under section 4 of TSCA. The objective of a long-term carcinogenicity study is to observe test animals for a major portion of their life span for development of neoplastic lesions during or after exposure to various doses of a test substance by an appropriate route of administration.

(b) *Source*. The source material used in developing this TSCA test guideline is the OPPTS harmonized test guideline 870.4200 (June 1996 Public Draft). This source is available at the address in paragraph (g) of this section.

(c) *Definitions*. The following definitions apply to this section.

Carcinogenicity is the development of neoplastic lesions as a result of the repeated daily exposure of experimental animals to a chemical by the oral, dermal, or inhalation routes 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 or its metabolites in susceptible tissues.

Dose in a carcinogenicity study is the amount of test substance administered via the oral, dermal or inhalation routes for a period of up to 24 months. Dose is expressed as weight of the test substance (grams, milligrams) per unit body weight of test animal (milligram per kilogram), or as weight of the test substance in parts per million (ppm) in food or drinking water. When exposed via inhalation, dose is expressed as weight of the test substance per unit volume of air (milligrams per liter) or as parts per million.

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

(d) *Test procedures*—(1) *Animal selection*—(i) *Species and strain*. Testing shall be performed on two mammalian species. Rats and mice are the species of choice because of their relatively short life spans, limited cost of maintenance, widespread use in pharmacological and toxicological studies, susceptibility to tumor induction, and the availability of inbred or sufficiently characterized strains. Commonly used laboratory strains shall be used. If other mammalian species are used, the tester shall provide justification/reasoning for their selection.

(ii) *Age/weight*. (A) Testing shall 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 shall not exceed $\pm 20\%$ of the mean weight for each sex.

(D) Studies using prenatal or neonatal animals may be recommended under special conditions.

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

(B) Females shall be nulliparous and nonpregnant.

(iv) *Numbers*. (A) At least 100 rodents (50 males and 50 females) shall be used at each dose level and concurrent control group.

(B) If interim sacrifices are planned, the number shall be increased by the number of animals scheduled to be sacrificed during the course of the study.

(C) 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% at 15 months in mice and 18 months in rats. Survival in any group should not fall below 25% at 18 months in mice and 24 months in rats.

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

(E) Each animal shall be assigned a unique identification number. Dead

animals, their preserved organs and tissues, and microscopic slides shall be identified by reference to the unique numbers assigned.

(v) *Husbandry*. (A) Animals may be group-caged by sex, but the number of animals per cage must not interfere with clear observation of each animal. The biological properties of the test substance or toxic effects (e.g., morbidity, excitability) may indicate a need for individual caging. Animals should be housed individually in dermal studies and during exposure in inhalation studies.

(B) The temperature of the experimental animal rooms should be at 22 ± 3 °C.

(C) The relative humidity of the experimental animal rooms should be 30 to 70%.

(D) Where lighting is artificial, the sequence should be 12 h light/12 h dark.

(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) Where necessary, the test substance is dissolved or suspended in a suitable vehicle. If a vehicle or diluent is needed, it should not elicit toxic effects itself. It is recommended that wherever possible the use of an aqueous solution be considered first, followed by consideration of solution in oil, and finally solution in other vehicles.

(ii) One lot of the test substance should be used, if possible, throughout the duration of the study, 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 of the test compound, and, if

possible, the name and quantities of contaminants and impurities.

(iii) If the test or control substance is to be incorporated into feed or another vehicle, the period during which the test substance is stable in such a mixture should be determined prior to the initiation of the study. Its homogeneity and concentration should be determined prior to the initiation of the study and periodically during the study. Statistically randomized samples of the mixture should be analyzed to ensure that proper mixing, formulation, and storage procedures are being followed, and that the appropriate concentration of the test or control substance is contained in the mixture.

(3) *Control groups*. A concurrent control group (50 males and 50 females) is required. This group shall be untreated or if a vehicle is used in administering the test substance, a vehicle control group. If the toxic properties of the vehicle are not known, both untreated and vehicle control groups are required.

(4) *Dose levels and dose selection*. (i) For risk assessment purposes, at least three dose levels shall 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 dose level should elicit signs of toxicity without substantially altering the normal life span due to effects other than tumors. The highest dose should be determined based on the findings from a 90-day study to ensure that the dose used is adequate to assess the carcinogenic potential of the test substance. Thus, the selection of the highest dose to be tested is dependent upon changes observed in several toxicological parameters in subchronic studies. The highest dose tested need not exceed 1,000 mg/kg/day.

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

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

(v) For skin carcinogenicity studies, when toxicity to the skin is a determining factor, the highest dose selected should not destroy the functional integrity of the skin, the intermediate dose should be a minimally irritating dose, and the low dose should be the highest nonirritating dose.

(vi) The criteria for selecting the dose levels for skin carcinogenicity studies, based on gross and histopathologic dermal lesions, are as follows:

(A) Gross criteria for reaching the high dose:

- (1) Erythema (moderate).
- (2) Scaling.
- (3) Edema (mild).
- (4) Alopecia.
- (5) Thickening.

(B) Histologic criteria for reaching the high dose:

- (1) Epidermal hyperplasia.
- (2) Epidermal hyperkeratosis.
- (3) Epidermal parakeratosis.
- (4) Adnexal atrophy/hyperplasia.
- (5) Fibrosis.
- (6) Spongiosis (minimal-mild).
- (7) Epidermal edema (minimal-mild).
- (8) Dermal edema (minimal-moderate).

(9) Inflammation (moderate).

(C) Gross criteria for exceeding the high dose:

- (1) Ulcers, fissures.
- (2) Exudate/crust (eschar).
- (3) nonviable (dead) tissues.
- (4) Anything leading to destruction of the functional integrity of the epidermis (e.g., caking, fissuring, open sores, eschar).

(D) Histologic criteria for exceeding the high dose:

- (1) Crust (interfollicular and follicular).
- (2) Microulcer.
- (3) Degeneration/necrosis (mild to moderate).
- (4) Epidermal edema (moderate to marked).
- (5) Dermal edema (marked).
- (6) Inflammation (marked).
- (5) *Administration of the test substance.*

The three main routes of administration are oral, dermal, and inhalation. The choice of the route of administration depends upon the physical and chemical characteristics of the test

substance and the form typifying exposure in humans.

(i) *Oral studies.* If the test substance is administered by gavage, the animals are dosed with the test substance on a 7-day per week basis for a period of at least 18 months for mice and hamsters and 24 months for rats. However, based primarily on practical considerations, dosing by gavage or via a capsule on a 5-day per week basis is acceptable. If the test substance is administered in the drinking water or mixed in the diet, then exposure should be on a 7-day per week basis.

(ii) *Dermal studies.* (A) The animals should be treated with the test substance for at least 6 h/day on a 7-day per week basis for a period of at least 18 months for mice and hamsters and 24 months for rats. However, based primarily on practical considerations, application on a 5-day per week basis is acceptable. Dosing should be conducted at approximately the same time each day.

(B) Fur should be clipped weekly from the dorsal area of the trunk of the test animals. Care should be taken to avoid abrading the skin which could alter its permeability. A minimum of 24 hrs should be allowed for the skin to recover before the next dosing of the animal.

(C) Preparation of test substance. Liquid test substances are generally used undiluted, except as indicated in paragraph (e)(4)(vi) of this section. Solids should be pulverized when possible. The substance should be moistened sufficiently with water or, when necessary, with a suitable vehicle to ensure good contact with the skin. When a vehicle is used, the influence of the vehicle on toxicity of, and penetration of the skin by, the test substance should be taken into account. The volume of application should be kept constant, e.g. less than 100 μ L for the mouse and less than 300 μ L for the rat. Different concentrations of test solution should be prepared for different dose levels.

(D) The test substance shall be applied uniformly over a shaved area which is approximately 10 percent of the total body surface area. In order to dose approximately 10 percent of the body surface, the area starting at the

scapulae (shoulders) to the wing of the ileum (hipbone) and half way down the flank on each side of the animal should be shaved. With highly toxic substances, the surface area covered may be less, but as much of the area as possible should be covered with as thin and uniform a film as practical.

(iii) *Inhalation studies.* (A) 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 18 months in mice and 24 months in rats. However, based primarily on practical considerations, exposure for 6 h/day on a 5-day per week basis is acceptable.

(B) The animals shall be tested in dynamic inhalation equipment designed to sustain a minimum air flow of 10 air changes per hr, an adequate oxygen content of at least 19%, 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.

(C) The selection of a dynamic inhalation chamber should be appropriate for the test substance and test system. Where a whole body chamber is used to expose animals to an aerosol, 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 shall not exceed 5% of the volume of the test chamber. It is recommended, but not required, that nose-only or head-only exposure be used for aerosol studies in order to minimize oral exposures due to animals licking compound off their fur. Heat stress to the animals should be minimized.

(D) The temperature at which the test is performed should be maintained at 22 ± 2 °C. The relative humidity should be maintained between 40 to 60%, but in certain instances (e.g., tests of aerosols, use of water vehicle) this may not be practicable.

(E) The rate of air flow shall be monitored continuously but recorded at least three times during exposure.

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

(G) The actual concentration of the test substance shall be measured in the breathing zone. During the exposure period, the actual concentrations of the test substance should be held as constant as practicable, monitored continuously or intermittently depending on the method of analysis. Chamber concentrations may be measured using gravimetric or analytical methods as appropriate. If trial run measurements are reasonably consistent (plus or minus 10 percent for liquid aerosol, gas, or vapor; plus or minus 20 percent for dry aerosol), the two measurements should be sufficient. If measurements are not consistent, then three to four measurements should be taken.

(H) During the development of the generating system, particle size analysis shall be performed to establish the stability of aerosol concentrations with respect to particle size. Measurement of aerodynamic particle size in the animals's breathing zone should be measured during a trial run. If median aerodynamic diameter (MMAD) values for each exposure level are within 10% of each other, then two measurements during the exposures should be sufficient. If pretest measurements are not within 10% of each other, three to four measurements should be taken. The MMAD particle size range should be between 1-3 μm . The particle size of hygroscopic materials should be small enough to allow pulmonary deposition once the particles swell in the moist environment of the respiratory tract.

(I) Feed shall be withheld during exposure. Water may also be withheld during exposure.

(6) *Observation period.* It is necessary that the duration of the carcinogenicity study comprise the majority of the normal life span of the strain of animals used. This time period shall not be less than 24 months for rats and 18 months for mice, and ordinarily not longer than 30 months for rats and 24 months for mice. For longer time periods, and where any other species are used, consultation with the Agency in regard to the duration of the study is advised.

(7) *Observation of animals.* (i) Observations shall be made at least once 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).

(ii) A careful clinical examination shall 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 activity, gait and posture, reactivity to handling or sensory stimuli, altered strength and stereotypes or bizarre behavior (e.g., self-mutilation, walking backwards).

(iii) Body weights shall 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) Measurements of feed consumption should be determined weekly during the first 13 weeks of the study and at approximately monthly intervals thereafter unless health status or body weight changes dictate otherwise. Measurement of water consumption should be determined at the same intervals if the test substance is administered in the drinking water.

(v) Moribund animals shall 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 shall be sacrificed.

(8) *Clinical pathology.* At 12 months, 18 months, and at terminal sacrifice, a blood smear shall be obtained from all animals. A differential blood count should be performed on blood smears from those animals in the highest dosage group and the controls from the terminal sacrifice. If these data, or data from the pathological examination indicate a need, then the 12- and 18-month blood smears should also be

examined. Differential blood counts should be performed for the next lower groups if there is a major discrepancy between the highest group and the controls. If clinical observations suggest a deterioration in health of the animals during the study, a differential blood count of the affected animals shall be performed.

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

(ii) At least the liver, kidneys, adrenals, testes, epididymides, ovaries, uterus, spleen, brain, and heart should be weighed wet as soon as possible after dissection to avoid drying. The lungs should be weighed if the test substance is administered by the inhalation route. 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) The following organs and tissues, or representative samples thereof, shall be preserved in a suitable medium for possible future histopathological examination.

(A) Digestive system.

(1) Salivary glands.

(2) Esophagus.

(3) Stomach.

(4) Duodenum.

(5) Jejunum.

(6) Ileum.

(7) Cecum.

(8) Colon.

(9) Rectum.

(10) Liver.

(11) Pancreas.

(12) Gallbladder (mice).

(B) Nervous system.

(1) Brain (multiple sections).

(2) Pituitary.

(3) Peripheral nerves.

(4) Spinal cord (three levels).

(5) Eyes (retina, optic nerve).

(C) Glandular system.

(1) Adrenals.

(2) Parathyroids.

(3) Thyroids.

(D) Respiratory system.

(1) Trachea.

(2) Lung.

(3) Pharynx.

(4) Larynx.

(5) Nose.

Environmental Protection Agency

§ 799.9420

(E) Cardiovascular/hematopoietic system.

(1) Aorta (thoracic).

(2) Heart.

(3) Bone marrow.

(4) Lymph nodes.

(5) Spleen.

(F) Urogenital system.

(1) Kidneys.

(2) Urinary bladder.

(3) Prostate.

(4) Testes/epididymides.

(5) Seminal vesicles.

(6) Uterus.

(7) Ovaries.

(8) Female mammary gland.

(G) Other.

(1) Skin.

(2) All gross lesions and masses.

(iv) In inhalation studies, the entire respiratory tract, including nose, pharynx, larynx, and paranasal sinuses should be examined and preserved. In dermal studies, skin from treated and adjacent control skin sites should be examined and preserved.

(v) Inflation of lungs and urinary bladder 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.

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

(10) *Histopathology.* (i) The following histopathology shall be performed:

(A) Full histopathology on the organs and tissues under paragraph (d)(9)

(iii) of this section of all animals in the control and high dose groups and all animals that died or were killed during the study.

(B) All gross lesions in all animals.

(C) Target organs 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 dose levels shall be examined as described in paragraph (d)(10)(i) of this section.

(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.

(e) *Data and reporting*—(1) *Treatment of results.* (i) Data shall 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) shall be evaluated by an appropriate statistical method. Any generally accepted statistical methods may be used; the statistical methods including significance criteria shall be selected during the design of the study.

(2) *Evaluation of study results.* (i) The findings of a 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 shall 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) In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailability of the test substance should be considered.

(iii) In order for a negative test to be acceptable, it must meet the following criteria: No more than 10% of any group is lost due to autolysis, cannibalism, or management problems; and survival in each group is no less than 50% at 15 months for mice and 18 months for rats. Survival should not fall below 25% at 18 months for mice and 24 months for rats.

(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, the following specific information shall be reported. Both individual and summary data should be presented.

(A) Test substance characterization should include:

- (1) Chemical identification.
- (2) Lot or batch number.
- (3) Physical properties.
- (4) Purity/impurities.
- (5) Identification and composition of any vehicle used.

(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.
- (4) Identification of animal diet.
- (5) Acclimation period.

(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.
- (4) *Test results*—(i) *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.

(ii) *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 clinical pathology and immunotoxicity screen when performed.

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

(G) Detailed description of all histopathological findings.

(H) Statistical treatment of results where appropriate.

(I) Historical control data.

(J) Achieved dose (mg/kg/day) as a time-weighted average if the test substance is administered in the diet or drinking water.

(iii) *Inhalation studies.* In addition, for inhalation studies the following shall be reported:

(A) *Test conditions.* The following exposure conditions shall be reported.

(1) Description of exposure apparatus including design, type, dimensions, source of air, system for generating particulate 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 particulate aerosol concentrations and size should be described.

(B) *Exposure data.* These shall 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) Particle size distribution, calculated MMAD and geometric standard deviation (GSD).

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

(f) *Quality assurance.* A system shall be developed and maintained to assure and document adequate performance of laboratory staff and equipment. The study shall be conducted in compliance with 40 CFR part 792—Good Laboratory Practice Standards.

(g) *References.* For additional background information on this test guideline, the following references should be consulted. These references are available for inspection at the TSCA Non-confidential Information Center, Rm. NE-B607, Environmental Protection Agency, 401 M St., SW., Washington, DC, 12 noon to 4 p.m., Monday through Friday, except legal holidays.

(1) Benitz, K.F. Ed. Paget, G.E. Measurement of Chronic Toxicity. *Methods of Toxicology* (Blackwell, Oxford, 1970) pp. 82-131.

(2) Fitzhugh, O.G. Chronic Oral Toxicity, Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Association of Food and Drug Officials of the United States. pp. 36-45 (1959, 3rd Printing 1975).

(3) Goldenthal, E.I. and D'Aguanno, W. Evaluation of Drugs, Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. The Association of Food and Drug Officials of the United States. pp. 60-67 (1959, 3rd Printing 1975).

(4) Organisation for Economic Co-operation and Development. Guidelines for Testing of Chemicals, Section 4-Health Effects, Part 451 Carcinogenicity Studies (Paris, 1981).

(5) Page, N.P. Chronic Toxicity and Carcinogenicity Guidelines. *Journal of Environmental Pathology and Toxicology*. 11:161-182 (1977).

(6) Page, N.P. Eds. Kraybill and Mehlman. Concepts of a Bioassay Program in Environmental Carcinogenesis. Vol.3. *Advances in Modern Toxicology* (Hemisphere, Washington, DC., 1977) pp. 87-171.

(7) Sontag, J.M. *et al.* Guidelines for Carcinogen Bioassay in Small Rodents. NCI-CS-TR-1 United States Cancer Institute, Division of Cancer Control and Prevention, Carcinogenesis Bioassay Program (Bethesda, MD).

[62 FR 43824, Aug. 15, 1997, as amended at 64 FR 35078, June 30, 1999]

§ 799.9430 TSCA combined chronic toxicity/carcinogenicity.

(a) *Scope.* This section is intended to meet the testing requirements under section 4 of the Toxic Substances Control Act (TSCA). The objective of a combined chronic toxicity/carcinogenicity study is to determine the ef-

fects of a substance in a mammalian species following prolonged and repeated exposure. The application of this section 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 effects and a determination of the carcinogenic potential as well as general toxicity, including neurological, physiological, biochemical, and hematological effects and exposure-related morphological (pathology) effects.

(b) *Source.* The source material used in developing this TSCA test guideline is the Office of Prevention, Pesticides, and Toxic Substances (OPPTS) harmonized test guideline 870.4300 (August 1998, final guideline). This source is available at the address in paragraph (h) of this section.

(c) *Definitions.* The following definitions apply to this section.

Carcinogenicity is the development of neoplastic lesions as a result of the repeated daily exposure of experimental animals to a chemical by the oral, dermal, or inhalation routes of exposure.

Chronic toxicity is the adverse effects occurring as a result of the repeated daily exposure of experimental animals to a chemical by the oral, dermal, or inhalation routes 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 or its metabolites in susceptible tissues.

Dose in a combined chronic toxicity/carcinogenicity study is the amount of test substance administered via the oral, dermal, or inhalation routes for a period of up to 24 months. Dose is expressed as weight of the test substance per unit body weight of test animal (milligrams per kilogram), or as weight of the test substance in parts per million (ppm) in food or drinking water. When exposed via inhalation, dose is expressed as weight of the test substance per unit volume of air (milligrams per liter) or as parts per million per day. For dermal application, dose is expressed as weight of the test substance (grams, milligrams) per unit