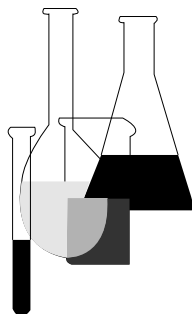




# Health Effects Test Guidelines OPPTS 870.3465 90-Day Inhalation Toxicity



## INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in title 40, chapter I, subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

**Final Guideline Release:** This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512-0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (<http://www.epa.gov/epahome/research.htm>) under the heading "Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines."

**OPPTS 870.3465 90-Day inhalation toxicity.**

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are 40 CFR 798.2450 Inhalation Toxicity; OPP 82-4 90-Day Inhalation—Rat (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09-82-025, 1982; and OECD 413 Subchronic Inhalation Toxicity: 90-Day.

(b) **Purpose.** In the assessment and evaluation of the toxic characteristics of a gas, volatile substance, or aerosol/particulate, determination of subchronic inhalation toxicity may be carried out after initial information on toxicity has been obtained by acute testing. The subchronic inhalation study has been designed to permit the determination of the no-observed-effect-level (NOEL) and toxic effects associated with continuous or repeated exposure to a test substance for a period of 90 days. This study is not capable of determining those effects that have a long latency period for development (e.g., carcinogenicity and life shortening). Extrapolation from the results of this study to humans is valid only to a limited degree. It can, however, provide useful information on health hazards likely to arise from repeated exposures by the inhalation route over a limited period of time. It will provide information on target organs and the possibilities of accumulation, and can be of use in selecting concentration levels for chronic studies and establishing safety criteria for human exposure. Hazards of inhaled substances are influenced by the inherent toxicity and by physical factors such as volatility and particle size.

(c) **Definitions.** The definitions in section 3 of the Toxic Substance Control Act (TSCA) and the definitions in 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. The following definitions also apply to this test guideline.

*Aerodynamic equivalent diameter* is defined as the diameter of a unit density sphere having the same terminal settling velocity as the particle in question, whatever its size, shape, and density. It is used to predict where in the respiratory tract such particles may be deposited.

*Concentration* in a subchronic inhalation study is the amount of test substance administered via inhalation for a period of 90 days. Concentration is expressed as weight of the test substance per unit volume of air (milligrams per liter or parts per million).

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

*Inhalable diameter* refers to that aerodynamic diameter of a particle which is considered to be inhalable for the organism. It is used to refer to particles which are capable of being inhaled and may be deposited anywhere within the respiratory tract

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

*Mass median aerodynamic diameter* (MMAD) is the median aerodynamic diameter and along with the geometric standard deviation (GSD) is used to describe the particle size distribution of any aerosol statistically based on the weight and size of the particles. Fifty percent of the particles by weight will be smaller than the median diameter and 50 percent of the particles will be larger.

*Subchronic inhalation toxicity* is the adverse effects occurring as a result of the repeated daily exposure of experimental animals to a chemical by inhalation for part (approximately 10 percent) of a life span.

(d) **Limit test.** If exposure at a concentration of 1 mg/L (expected human exposure may indicate the need for a higher concentration), or where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration produces no observable toxic effects, then a full study using three concentrations might not be necessary.

(e) **Test procedures—(1) Animal selection—(i) Species and strain.** A mammalian species shall be used for testing. A variety of rodent species may be used, although the rat is the preferred species. Commonly used laboratory strains should be employed. If another mammalian species is used, the tester shall provide justification/reasoning for its selection.

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

(B) Dosing of rodents should generally begin no later than 8–9 weeks of age.

(C) At the commencement of the study the weight variation of animals used shall not exceed  $\pm 20$  percent of the mean weight for each sex.

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

(B) Females shall be nulliparous and nonpregnant.

(iv) **Numbers.** (A) At least 20 animals (10 females and 10 males) should be used for each test group.

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

(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 shall be assigned a unique identification number. Dead animals, their preserved organs and tissues, and microscopic slides shall be identified by reference to the animal's unique number.

(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 must be housed individually in inhalation chambers during exposure to aerosols.

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

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

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

(E) Control and test animals should be fed from the same batch and lot. The feed should be analyzed to assure adequacy of nutritional requirements of the species tested and for impurities that might influence the outcome of the test. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

(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. An acclimation period of at least 5 days is recommended.

(2) **Control and test substances.** (i) Whenever it is necessary to formulate the test substance with a vehicle for aerosol generation, the vehicle ideally should not elicit toxic effects or substantially alter the chemical or toxicological properties of the test substance.

(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 substance and, if technically feasible, the name and quantities of unknown contaminants and impurities.

(3) **Control groups.** A concurrent control group is required. This group shall be an untreated or sham-treated control group. Except for treatment with the test substance, animals in the control group shall be handled in a manner identical to the test group animals. Where a vehicle other than water is used to generate a substance, a vehicle control group should be used. If the toxic properties of the vehicle are not known or cannot be made available, both untreated and vehicle control groups are required.

(4) **Satellite group.** A satellite group of 20 animals (10 animals per sex) may be treated with the high concentration level for 90 days and observed for reversibility, persistence, or delayed occurrence of toxic effects for a post-treatment period of appropriate length, normally not less than 28 days. In addition, a control group of 20 animals (10 animals of each sex) should be added to the satellite study.

(5) **Concentration levels and concentration selection.** (i) In sub-chronic toxicity tests, it is desirable to have a concentration-response relationship as well as a NOEL. Therefore, at least three concentration levels plus a control and, where appropriate, a vehicle control (corresponding to the concentration of vehicle at the highest exposure level) shall be used. Concentrations should be spaced appropriately to produce test groups with a range of toxic effects. The data should be sufficient to produce a concentration-response curve.

(ii) The highest concentration should result in toxic effects but not produce an incidence of fatalities which would prevent a meaningful evaluation.

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

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

(v) In the case of potentially explosive test substances, care should be taken to avoid generating explosive concentrations.

(6) **Administration of the test substance.** Animals should be exposed to the test substance for 6 h per day on a 7-day per week basis for a period of at least 90 days. Based primarily on practical considerations, exposure for 6 h per day on a 5-day per week basis is acceptable.

(7) **Observation period.** The animals should be observed for a period of 90 days. Animals in the satellite group (if used) scheduled for follow-up observations should be kept for at least 28 days further without treatment to assess reversibility.

(8) **Exposure specifications.** (i) The animals shall be tested in dynamic inhalation equipment designed to sustain a minimum airflow 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 the surrounding areas. It is not normally necessary to measure chamber oxygen concentration if airflow is adequate.

(ii) 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 percent 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 should be minimized.

(iii) The temperature at which the test is performed should be maintained at  $22 \pm 2$  °C. The relative humidity should be maintained between 40–60 percent, but in certain instances (e.g., use of water vehicle) this may not be practicable.

(9) **Physical measurements.** Measurements or monitoring shall be made of the following:

(i) The rate of airflow shall be monitored continuously but recorded at least three times during the exposure.

(ii) The actual concentrations of the test substance shall be measured in the animal's breathing zone. During the exposure period, the actual concentrations of the test substance shall be held as constant as practicable and monitored continuously or intermittently depending on the method of analysis. Chamber concentration may be measured using gravimetric or analytical methods as appropriate. If trial run measurements are reasonably consistent ( $\pm 10$  percent for liquid aerosol, gas, or vapor;  $\pm 20$  percent for dry aerosol), then two measurements should be sufficient. If measurements are not consistent, three to four measurements should be taken. Whenever the test substance is a formulation, or it is necessary to formulate the test substance with a vehicle for aerosol generation, the analytical concentration must be reported for the total formulation, and not just for the active ingredient (AI). If, for example, a formulation contains 10 percent AI and 90 percent inerts, a chamber analytical limit concentration of 2 mg/L would consist of 0.2 mg/L of the AI. It is not necessary to analyze inert ingredients provided the mixture at the animal's breathing zone is analogous to the formulation; the grounds for this conclusion must be provided in the study report. If there is some difficulty in measuring chamber analytical concentration due to precipitation, nonhomogeneous mixtures, volatile components, or other factors, additional analyses of inert components may be necessary.

(iii) 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. The MMAD particle size range should be between 1–3  $\mu\text{m}$ . The particle size of hygroscopic materials should be small enough when dry to assure that the size of the swollen particle will still be within the 1–3  $\mu\text{m}$  range. Measurements of aerodynamic particle size in the animal's breathing zone should be measured during a trial run. If MMAD values for each exposure level are within 10 percent of each other, then two measurements during the exposures should be sufficient. If pretest measurements are not within 10 percent of each other, three to four measurements should be taken.

(iv) Temperature and humidity shall be monitored continuously and recorded at least three times during an exposure.

(10) **Feed and water during exposure period.** Feed shall be withheld during exposure. Water may also be withheld during exposure.

(11) **Observation of animals.** (i) During and following exposure, observations are made and recorded systematically; individual records should be maintained for each animal. It is not always possible to observe animals during exposure in a whole-body chamber.

(ii) Observations shall be made at least twice each day for morbidity and mortality. Appropriate actions should be taken to minimize loss of animals to the study (e.g., Necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals).

(iii) General clinical observations should be made at least once a day, preferably at the same time each day, taking into consideration the peak period of anticipated effects after dosing. The clinical condition of the animal should be recorded.

(iv) A careful clinical examination should be made at least once prior to the initiation of treatment (to allow for within subject comparisons) and once weekly during treatment in all animals. These observations should be made outside the home cage, preferably in a standard arena, and at similar times on each occasion. Effort should be made to ensure that variations in the observation conditions are minimal. Observations should be detailed and carefully recorded, preferably using scoring systems, explicitly defined by the testing laboratory. Signs noted should include, but not be limited to, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backwards) should be recorded.



(v) Once, near the end of the exposure period and in any case not earlier than in week 11, assessment of motor activity, grip strength, and sensory reactivity to stimuli of different types (e.g., visual, auditory, and proprioceptive stimuli) should be conducted. Further details of the procedures that could be followed are described in the references listed under paragraphs (h)(3), (h)(4), (h)(5), (h)(7), (h)(8), and (h)(11) of this guideline.

(vi) Functional observations conducted towards the end of the study may be omitted when data on functional observations are available from other studies and the daily clinical observations did not reveal any functional deficits.

(vii) Exceptionally, functional observations may be omitted for groups that otherwise reveal signs of toxicity to an extent that would significantly interfere with functional test performance.

(viii) Individual weights of animals shall be determined shortly before the test substance is administered, and weekly thereafter.

(ix) Food consumption shall also be determined weekly if abnormal body weight changes are observed.

(x) Moribund animals should be removed and sacrificed when noticed and the time of death should be recorded as precisely as possible.

(xi) At termination, all survivors in the treatment groups shall be sacrificed.

(12) **Clinical pathology.** Hematology and clinical chemistry examinations should be made on all animals, including controls, of each sex in each group. The hematology and clinical chemistry parameters should be examined at terminal sacrifice at the end of the study. Overnight fasting of the animals prior to blood sampling is recommended. Overall, there is a need for a flexible approach in the measures examined, depending on the observed or expected effects from a chemical, and in the frequency of measures, depending on the duration of potential chemical exposures.

(i) Hematology. The recommended parameters are red blood cell count, hemoglobin concentration, hematocrit, 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 activated partial 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 2 hepatic enzymes, (such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, or gamma glutamyl transpeptidase) should also be measured. Measurements of additional enzymes (of hepatic or other origin) and bile acids, may also be useful.

(C) If a test chemical has an effect on the hematopoietic system, reticulocyte counts and bone marrow cytology may be indicated.

(D) Other determinations that should be carried out if the test chemical is known or suspected of affecting related measures include calcium, phosphorus, fasting triglycerides, hormones, methemoglobin, and cholinesterases.

(iii) Optionally, the following urinalysis determinations could be performed during the last week of the study using timed urine volume collection: appearance, volume, osmolality or specific gravity, pH, protein, glucose, and blood/blood cells.

(13) **Ophthalmological examination.** Ophthalmological examinations using an ophthalmoscope or an equivalent device should be made on all animals prior to the administration of the test substance and on all high dose and control groups at termination. If changes in the eyes are detected, all animals in the other dose groups should be examined.

(14) **Gross necropsy.** (i) All animals shall be subjected to a full gross necropsy which includes examination of the external surface of the body, all orifices and the cranial, thoracic, and abdominal cavities and their contents.

(ii) The liver, kidneys, adrenals, testes, epididymides, ovaries, uterus, thymus, spleen, brain, and heart shall be trimmed and weighed wet, as soon as possible after dissection to avoid drying.

(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 (where present).
- (B) Nervous system.
- (1) Brain (including sections of medulla/pons, cerebellum, and cerebrum).
  - (2) Pituitary.
  - (3) Peripheral nerve (sciatic or tibial, preferably in close proximity to the muscle).
  - (4) Spinal cord (three levels: cervical, mid-thoracic, and lumbar).
  - (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.
- (E) Cardiovascular/hematopoietic system.
- (1) Aorta.
  - (2) Heart.
  - (3) Bone marrow (and/or fresh aspirate).

(4) Lymph nodes (preferably one node covering the route of administration and another one distant from the route of administration).

(5) Spleen.

(6) Thymus.

(F) Urogenital system.

(1) Kidneys.

(2) Urinary bladder.

(3) Prostate.

(4) Testes.

(5) Epididymides.

(6) Seminal vesicle(s).

(7) Uterus.

(8) Ovaries.

(9) Female mammary gland.

(G) Other.

(1) Lacrimal gland.

(2) Skin.

(3) All gross lesions and masses.

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

(A) Full histopathology on the respiratory tract and other organs and tissues, listed under paragraph (e)(15)(iii) of this guideline, of all animals in the control and high exposure 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.

(D) Lungs of all animals. Special attention to examination of the respiratory tract should be made for evidence of infection as this provides a convenient assessment of the state of health of the animals.

(E) When a satellite group is used, histopathology shall be performed on tissues and organs identified as showing effects in the treated groups.

(ii) If excessive early deaths or other problems occur in the high exposure group compromising the significance of the data, the next concentration should be examined for complete histopathology.

(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.

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

(2) **Evaluation of study results.** The findings of the subchronic inhalation toxicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the observed toxic effects and the necropsy and histopathological findings. The evaluation will include the relationship between the concentration of the test substance and duration of exposure, and the presence or absence, the 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. A properly conducted subchronic test should provide a satisfactory estimation of a no-effect level. It also can indicate the need for an additional longer-term study and provide information on the selection of concentrations.

(3) **Test report.** In addition to reporting requirements specified under EPA Good Laboratory Practice Standards, 40 CFR part 792, subpart J and 40 CFR part 160, and the OECD principles of GLP (ISBN 92-64-12367-9) the following specific information shall be reported:

(i) Test substance characterization shall include:

(A) Chemical identification.

(B) Lot or batch number.

(C) Physical properties.

(D) Purity/impurities.

(E) Identification and composition of any vehicle used.

(ii) Test system information shall include:

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

(B) Age, sex, and body weight data.

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

(D) Identification of animal diet.

(E) Acclimation period.

(iii) Test procedure information shall include:

(A) Method of randomization used.

(B) Full description of experimental design and procedure.

(C) Exposure regimen including levels, methods, and volume.

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

(1) Description of exposure apparatus including design, type, volume, source of air, system for generating 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.

(E) 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 mass median aerodynamic diameter (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 guidelines.

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

- (1) Number of animals exposed.
- (2) Number of animals showing signs of toxicity.
- (3) Number of animals dying.

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

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

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

- (3) Body weight data.
- (4) Feed consumption data, when collected.
- (5) Results of ophthalmological examination.
- (6) Results of hematological tests performed. .
- (7) Results of clinical chemistry tests performed.
- (8) Results of urinalysis, if performed.
- (9) Results of observations made.

(10) Necropsy findings, including absolute and relative organ weight data.

(11) Detailed description of all histopathological findings.

(12) Statistical treatment of results, where appropriate.

(g) **Quality control.** A system shall be developed and maintained to assure and document adequate performance of laboratory staff and equipment. The study must be conducted in compliance with GLP regulations.

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

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- (4) Gad S.C. A Neuromuscular Screen for Use in Industrial Toxicology. *Journal of Toxicology and Environmental Health.* 9, 691–704. (1982)
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- (6) MacFarland, H.N. Respiratory Toxicology, Essays in Toxicology. Ed. W.J. Hayes. Vol. 7 (New York: Academic Press, 1976) pp. 121–154.
- (7) Meyer O.A., Tilson H.A., Byrd W.C., Riley M.T. A Method for the Routine Assessment of Fore- and Hind-Limb Grip Strength of Rats and Mice. *Neurobehav. Toxicol.* 1, 233–236. (1979)
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- (9) National Academy of Sciences. Principles and Procedures for Evaluating the Toxicity of Household Substances, a report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).
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- (13) U.S. Environmental Protection Agency, Office of Pesticide Programs, Health Effects Division. Interim policy for particle size and limit concentration issues in inhalation toxicity studies (February 1, 1994).



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

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

(16) World Health Organization. Principles for pre-clinical testing of drug safety. *WHO Technical Report Series No. 341* (Geneva: World Health Organization, 1966).