Environmental Protection Agency § 799.9130

(10) A list of references cited in the body of the report. References to any published literature used in developing the test protocol, performing the testing, making and interpreting observations, and compiling and evaluating the results.

(f) 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., NW., Washington, DC, 12 noon to 4 p.m., Monday through Friday, except legal holidays.


[55 FR 78774, Dec. 15, 2000]

§ 799.9130 TSCA acute inhalation toxicity.

(a) Scope. This section is intended to meet testing requirements under section 4 of the Toxic Substances Control Act (TSCA). Determination of acute toxicity is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance that may be inhaled such as a gas, volatile substance, or aerosol/particle. It provides information on health hazards likely to arise from short-term exposure by the inhalation route. Data from an acute study may serve as a basis for classification and labeling. It is traditionally a step in establishing a dosage regimen in subchronic and other studies and may provide initial information on the mode of toxic action of a substance. An evaluation of acute toxicity data should include the relationship, if any, between the animals' exposure to the test substance and the incidence and severity of all abnormalities, including behavioral and clinical abnormalities, the reversibility of observed abnormalities, gross lesions, body weight changes, effects on mortality, and any other toxic effects.

(b) Source. The source material used in developing this TSCA test guideline is the harmonized Office of Prevention, Pesticides, and Toxic Substances (OPPTS) test guideline 870.1300 (August 1998, final guideline). These sources are available at the address in paragraph (g) of this section.

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

Acute inhalation toxicity is the adverse effect caused by a substance following a single uninterrupted exposure by inhalation over a short period of time (24 hours or less) to a substance capable of being inhaled.

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 is expressed as weight of the test substance per unit volume of air, e.g., milligrams per liter. Geometric standard deviation (GSD) is a dimensionless number equal to the...
ratio between the mass median aero-
dynamic diameter (MMAD) and either 84% or 16% of the diameter size dis-
tribution (e.g., MMAD = 2 μm; 84% = 4 μm;
GSD = 4/2 = 2.0.) The MMAD, together
with the GSD, describe the particle size
distribution of an aerosol. Use of the
GSD may not be valid for non-
lognormally distributed aerosols. (If
the size distribution deviates from the
lognormal, it shall be noted).

Inhalable diameter refers to that aero-
dynamic diameter of a particle which
is considered to be inhalable for the or-
ganism under study. It is used to refer
to particles which are capable of being
inhaled and deposited anywhere within the
respiratory tract.

LC₅₀ (median lethal concentration) is
a statistically derived estimate of a
concentration of a substance that can
be expected to cause death during expo-
sure or within a fixed time after expo-
sure in 50% of animals exposed for a
specified time. The LC₅₀ value is a rel-
atively coarse measurement useful
only for classification and labeling pur-
poses and an expression of the lethal
potential of the test substance fol-
lowing inhalation. The LC₅₀ value is ex-
pressed as weight of test substance per
unit volume of air (milligrams per
liter) or parts per million. For clarity,
the exposure duration and test animal
species should also be specified, e.g., 4
hours LC₅₀ in F344.

Mass median aerodynamic diameter
(MMAD) is the median aero-dynamic
diameter and, along with the geometric
standard deviation, 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% of the particles will be larger.

(d) Approaches to the determination of
acute toxicity. (1) EPA recommends the
following means to reduce the number
of animals used to evaluate acute ef-
fects of chemical exposure while pre-
serving its ability to make reasonable
judgments about safety:

(i) Using data from substantially
similar mixtures. In order to minimize
the need for animal testing, the Agen-
cy encourages the review of existing
acute toxicity information on mixtures
that are substantially similar to mix-
tures under investigation. In certain
cases, it may be possible to get enough
information to make preliminary haz-
ard evaluations that may reduce the
need for further animal testing.

(ii) Limit test. When data on structur-
ally related chemicals are inade-
quate, a limit test may be considered.
In the limit test, a single group of five
males and five females is exposed to 2
mg/L for 4 hours, or where this is not
possible due to physical or chemical
properties of the test substance, the
maximum attainable concentration
where a particle size distribution hav-
ing an MMAD between 1 and 4 μm can-
ot be maintained, using the proce-
dures described under paragraph (e) of
this section. For fibers, the bivariate
distribution of length and diameter
must ensure inhalability. For gases
and vapors, the concentrations need
not be greater than 50,000 ppm or 50%
of the lower explosive limit, whichever
is lower. If a test at an aerosol or par-
ticulate exposure of 2 mg/L (actual con-
centration of respirable substance) for
4 hours or, where this is not feasible,
the maximum attainable concentra-
tion, using the procedures described for
this study, produces no observable
toxic effects, then a full study using
three concentrations will not be nec-
essary. Similarly, if a test at a gas or
vapor exposure of 50,000 ppm or 50%
of the lower explosive limit, whichever is
lower, produces no observable toxic ef-
facts, then a full study using three con-
centrations will not be necessary.

(2) [Reserved]

(e) Conventional acute toxicity test—(1)
Principle of the test method. Several
groups of experimental animals are ex-
posed to the test substance in gradu-
ated concentrations for a defined pe-
riod, one concentration being used per
group. When a vehicle other than water
is used to help generate an appropriate
concentration of the substance in the
atmosphere, a vehicle control group
should be used when historical data are
not available or adequate to determine
the acute inhalation toxicity of the ve-
hicle. Subsequently, observations of ef-
facts and death are made. Animals that
die during the test are necropsied and
at the conclusion of the test surviving
animals are sacrificed and necropsied.
This guideline is directed primarily to
studies in rodent species but may be adapted for studies in non-rodents. Animals showing severe and enduring signs of distress and pain may need to be sacrificed. Dosing test substances in a way known to cause marked pain and distress due to corrosive or irritating properties need not be carried out.

(2) **Substance to be tested.** Test, control, and reference substances are discussed under EPA Good Laboratory Practice Standards at 40 CFR part 792, subpart f.

(3) **Test procedures**—(i) **Preparation.** Healthy young adult animals are acclimatized to the laboratory conditions for at least 5 days prior to the test. Before the test, animals are randomized and assigned to the required number of groups.

(ii) **Animal selection**—(A) **Species and strain.** (1) Although several mammalian test species may be used, the preferred species is the rat. Commonly used laboratory strains should be employed. If another mammalian species is used, the investigator should provide justification and reasoning for the selection.

(2) **Health Status.** Body weight and feed consumption are not sufficient indicators of the health status of animals prior to initiating an inhalation toxicity study. Prior to initiating the study, animals must be monitored for known viral and bacterial respiratory pathogens determined by conventional microbiological assays (e.g., serology). The animals must be free from pathogens at the start of exposure.

(B) **Age.** Young adult rats between 8–12 weeks old at the beginning of dosing, should be used. The weight variation in animals or between groups used in a test should not exceed ±20% of the mean weight of each sex.

(C) **Number of animals and sex.** (1) At least five experimentally naive animals are used at each concentration and they must be of one sex. After completion of the study in one sex, at least one group of five animals of the other sex is exposed to establish that animals of this sex are not markedly more sensitive to the test substance. The use of fewer animals may be justified in individual circumstances. Where adequate information is available to demonstrate that animals of the sex tested are markedly more sensitive, testing in animals of the other sex is not required. An acceptable option would be to test at least one group of five animals per sex at one or more dose levels to definitively determine the more sensitive sex prior to conducting the main study.

(2) **Females must be nulliparous and nonpregnant.**

(3) **In acute toxicity tests with animals of a higher order than rodents,** the use of fewer animals per concentration group should be considered.

(D) **Assignment of animals.** (1) Each animal must be assigned a unique identification number. A system to assign animals to test groups and control groups randomly is required.

(2) **Control groups.** A concurrent untreated control group is not necessary. Where a vehicle other than water is used to generate an appropriate concentration of the test substance in the atmosphere and historical data are not available or adequate to determine the acute toxicity of the vehicle, a vehicle control group must be used. The vehicle control group must be a sham-treated group. Except for treatment with the test substance, animals in the vehicle control group must be handled in a manner identical to the test-group animals.

(E) **Housing.** The animals may be group-caged by sex, but the number of animals per cage should not exceed 120% of the mean weight of each sex.

(F) **Inhalation equipment.** (1) Animals can be exposed to the substance by either a nose-only procedure or in a
whole-body exposure chamber. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas. The nose-only exposure procedure is recommended for studies of aerosols to minimize exposures confounding resultant from test substance ingestion due to test animal fur licking following exposures. Animals must be acclimated to the nose-only exposure chamber prior to study and heat stress minimized during testing.

(2) Inhalation chambers. The animals must be tested in inhalation equipment designed to sustain a dynamic airflow for nose-only exposures of at least 300 ml/minute/animal or an airflow for whole-body exposures of at least 12 to 15 air changes per hour and ensure an adequate oxygen content of at least 19% and an evenly distributed exposure atmosphere. Where a whole-body chamber is used, its design must minimize crowding by providing individual caging. As a general rule, to ensure stability of a chamber atmosphere, the total “volume” of the test animals should not exceed 5% of the volume of the test chamber.

(3) Environmental conditions. The temperature at which the test is performed must be maintained at 22 °C (±2 °C). Ideally, the relative humidity should be maintained between 40% and 60%, but in certain instances (e.g., tests using water as a vehicle), this may not be practical.

(4) Physical measurements. Measurements or monitoring must be made of the following:

(1) Chemical purity of the test material must be analyzed. If the test substance is present in a mixture, the mass and composition of the entire mixture, as well as the principal compound, must be measured. If there is some difficulty in measuring chamber analytical concentration due to precipitation, nonhomogeneous mixtures, volatile components, or other factors, additional analyses of components may be necessary.

(2) The rate of air flow should be monitored continuously, and must be recorded at least every 30 minutes during the exposure period.

(3) The actual concentrations of the test substance must be measured in the breathing zone. During the exposure period, the actual concentrations of the test substance must be held as constant as practicable, monitored continuously or intermittently depending on the method of analysis, and recorded at least three times (i.e., at the beginning, at an intermediate time, and at the end) during the exposure period. Chamber concentration may be measured using gravimetric or analytical methods as appropriate. If trial run measurements are reasonably consistent (±10% for liquid aerosol, gas, or vapor; ±20% for dry aerosol), then a minimum of two measurements are sufficient. If measurements are not consistent, then a minimum of four measurements should be taken.

(4) During the development of the generating system, particle size analysis must be performed to establish the stability of aerosol concentrations. During exposure, analysis should be conducted as often as necessary to determine the consistency of particle size distribution. The MMAD particle size range should be between 1–4 μm. The particle size of hygroscopic materials must be small enough when dry to assure that the size of the swollen particle will still be within the 1–4 μm MMAD range. Characterization for fibers must include the bivariate distribution of length and diameter; this distribution must ensure inhalability. Measurements of aerodynamic particle size in the animal’s breathing zone must be measured during a trial run. If MMAD values for each exposure level are within 10% of each other, then a minimum of two measurements should be sufficient. If pretest measurements are not within 10% of each other, then a minimum of four measurements should be taken.

(5) Temperature and humidity must be monitored continuously, and must be recorded at least every 30 minutes.

(iii) Exposure duration and concentration levels. (A) Exposure duration. Shortly before exposure, the animals are weighed and then exposed to the test target concentration in the designated apparatus for 4 hour exposure period after equilibration of the chamber concentrations. The target concentration is defined by an average of 5% for gases and vapors and 15% for
particles and aerosols. The animals are weighed again at the conclusion of the exposure period to determine body weight change. Other durations may be needed to meet specific requirements. Food must be withheld during exposure. Water may also be withheld in certain circumstances.

(B) Exposure concentration levels. At least three concentration levels and a vehicle control group, if required (see paragraph (e)(3)(ii)(D)(2) of this section), must be used. The concentration levels should be spaced appropriately to produce a concentration-response curve and permit an estimation of the median lethal concentration (LC₅₀). The concentrations can either be linearly or logarithmically spaced depending on the anticipated steepness of the concentration-response curve. A rationale for concentration selection should be provided to indicate that the selected concentrations will maximally support detection of concentration-response relationship. The high concentration should be clearly toxic or a limit concentration, but should not result in an incidence of fatalities that would preclude a meaningful evaluation of the data. The lowest concentration should define a no-observed-effects level (NOEL). Range-finding studies using single animals may help to estimate the positioning of the test groups so that no more than three concentration levels will be necessary.

(C) When the physical and chemical properties of the test substance show a low flash point or the test substance is otherwise known or thought to be explosive, care must be taken to avoid exposure level concentrations that could result in an exposure chamber explosion during the test.

(iv) Observation period. The observation period must be at least 14 days. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, rate of onset, and length of recovery period, and thus may be extended when considered necessary. The time at which signs of toxicity appear, the duration of the signs observed, and the time of death must be recorded and are important, especially if there is a tendency for delayed effects.

(v) Observation of animals. (A) A careful clinical examination must be made at least once each day.

(B) Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study, e.g., necropsy or refrigeration of those animals found dead and isolation of weak or moribund animals.

(C) Observations must 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 stereotypies or bizarre behavior (e.g., self mutilation, walking backwards).

(D) Individual weights of animals must be determined pre-exposure and post-exposure, weekly after exposure, and at death. Changes in weights should be calculated and recorded when survival exceeds 1 day.

(E) The time of death should be recorded as precisely as possible.

(vi) Gross pathology. (A) At the end of the test, surviving animals must be weighed, sacrificed and a gross necropsy must be performed on all animals under test, with particular reference to any changes in the respiratory tract. All gross pathology changes must be recorded.

(1) The gross necropsy must include examination of orifices and the cranial, thoracic, and abdominal cavities, and contents.

(2) At least the lungs, liver, kidneys, adrenals, brain, and gonads should be weighed wet, as soon as possible after dissection to avoid drying.

(3) Optionally, the following organs and tissues, or representative samples thereof, may be preserved in a suitable medium for possible future histopathological examination: All gross lesions; brain-including sections of medulla/pons; cerebellar cortex and cerebral cortex; pituitary; thyroid/parathyroid; thymus; heart; sternum with bone marrow; salivary glands;
§ 799.9130  

Liver; spleen; kidneys; adrenals; pancreas; gonads; accessory genital organs (epididymis, prostate, and, if present, seminal vesicles); aorta; skin; gall bladder (if present); esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph nodes; thigh musculature; peripheral nerve; spinal cord at three levels cervical, midthoracic, and lumbar; and eyes. Respiratory tract tissues should be perfusion preserved in a suitable medium.

(B) If necropsy cannot be performed immediately after a dead animal is discovered during the observation period, the animal should be refrigerated (not frozen) at temperatures low enough to minimize autolysis. Necropsies should be performed as soon as possible after death (normally within 24 to 48 hours).

(vii) Additional evaluations. In animals surviving 24 hours or more, microscopic examination of organs showing evidence of gross pathology should be considered since it may yield useful information on the nature of acute toxic effects.

(f) Data and reporting—(1) Treatment of results. Data must be summarized in tabular form showing for each test group the number of animals at the start of the test, body weights, time of death of individual animals at different exposure levels, number of animals displaying other signs of toxicity, description of toxic effects and necropsy findings. The method used for calculation of the LC₅₀ or any other parameters must be specified and referenced. Some acceptable methods for parameter estimation are described in the references described in paragraphs (g)(1), (g)(2), and (g)(3) of this section.

(2) Evaluation of results. The LC₅₀ value should be considered in conjunction with the observed toxic effects and the necropsy findings. The evaluation should include the relationship, if any, between exposure of animals to the test substance and the incidence and severity of all abnormalities including behavioral and clinical abnormalities, gross lesions, body weight changes, mortality, and other toxic effects.

(3) Test report. In addition to the reporting requirements specified under EPA Good Laboratory Practice Standards at 40 CFR part 792, subpart J, the following specific information must be reported. The test report shall include:

(i) Test conditions. (A) Description of exposure apparatus including design, type, dimensions.

(B) Source of air, system for generating the test article as particle, aerosol, gas, or vapor.

(C) Method for conditioning air, equipment for measuring temperature, humidity, particle size or particulate aerosol concentration size, and actual concentration.

(D) Treatment of exhaust air and the method of housing the animals in a test chamber when this is used.

(ii) Exposure data. The exposure data must be tabulated and presented with mean values and a measure of variability (e.g., standard deviation) and should include:

(A) Chemical purity of the test material.

(B) Airflow rates through the inhalation equipment.

(C) Temperature and humidity of the air.

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

(E) Actual (analytical or gravimetric) concentration in test breathing zone.

(F) Particle size distribution (calculated MMAD and GSD) and the bivariate distribution of fiber length and diameter, where appropriate.

(G) 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 these aspects of this section.

(iii) Species, strain, sex, and source of test animals.

(iv) Method of randomization in assigning animals to test and control groups.

(v) Rationale for selection of species, if other than that recommended.

(vi) Results. Tabulation of individual and test group data by sex and exposure concentration level (e.g., number of animals exposed, number of animals showing signs of toxicity and number of animals that died or were sacrificed during the test).
(A) Description of toxic effects including time of onset, duration, reversibility, and relationship to the exposure concentration levels.
(B) Pre-exposure and post-exposure body weight change in animals, and weight change during the observation period.
(C) Time of dosing and time of death during or following exposure.
(D) Concentration-response curves for mortality and other toxic effects (when permitted by the method of determination).
(E) Gross pathology necropsy findings in the test animals and vehicle control animals, if included. Data must be tabulated to show the counts and incidence of gross alterations observed for each group tested and the number of animals affected by each type of lesion along with the location and frequency of each type of lesion.
(F) Histopathology findings and any additional evaluations (e.g., clinical chemistry), if performed.
(vii) Description of any pretest conditioning, including diet, quarantine and treatment for disease.
(viii) Description of caging conditions, including: number (or change in number) of animals per cage, bedding material, ambient temperature and humidity, photoperiod, and identification of diet of test animals.
(ix) Manufacturer (source), lot number, and purity of test substance.
(x) Identification and composition of any vehicles (e.g., diluents, suspending agents, and emulsifiers) or other materials, if used in administering the test substance.
(xi) A list of references cited in the body of the report. References to any published literature used in developing the test protocol, performing the testing, making and interpreting observations, and compiling and evaluating the results.

(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., NW., Washington, DC 12 noon to 4 p.m., Monday through Friday, except legal holidays.

(8) U. S. EPA. Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies. 21/94. Health Effects Division, Office of Pesticide Programs.

§ 799.9135 TSCA acute inhalation toxicity with histopathology.

(a) Scope. This section is intended to meet the testing requirements under section 4 of the Toxic Substances Control Act (TSCA). In the assessment and evaluation of the potential human health effects of chemical substances, it is appropriate to test for acute inhalation toxic effects. The goals of this test are to characterize the exposure-response relationship for sensitive endpoints following acute exposure and to characterize toxicologic response following acute high exposures. The