

changes, effects on mortality, and any other toxic effects. The LD₅₀ value should always be considered in conjunction with the observed toxic effects and any necropsy findings. The LD₅₀ value is a relatively coarse measurement, useful only as a reference value for classification and labeling purposes, and for an expression of the lethal potential of the test substance by the ingestion route. Reference should always be made to the experimental animal species in which the LD₅₀ value was obtained.

(C) *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:

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

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

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

(4) Tabulation of individual and test group data by sex and dose 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).

(i) Description of toxic effects, including their time of onset, duration, reversibility, and relationship to dose.

(ii) Body weights.

(iii) Time of dosing and time of death after dosing.

(iv) Dose-response curves for mortality and other toxic effects (when permitted by the method of determination).

(v) Gross pathology findings.

(vi) Histopathology findings and any additional clinical chemistry evaluations, if performed.

(5) Description of any pretest conditioning, including diet, quarantine and treatment for disease.

(6) 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.

(7) Manufacturer, source, purity, and lot number of test substance.

(8) Relevant properties of substance tested including physical state and pH (if applicable).

(9) Identification and composition of any vehicles (e.g., diluents, suspending agents, and emulsifiers) or other materials used in administering the test substance.

(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.

(1) Chanter, D.O. and Heywood, R. The LD₅₀ Test: Some Considerations of Precision. *Toxicology Letters* 10:303-307 (1982).

(2) Finney, D.J. Chapter 3—Estimation of the median effective dose and Chapter 4—Maximum likelihood estimation, *Probit Analysis*, 3rd ed. Cambridge, London (1971).

(3) Finney, D.J. The Median Lethal Dose and Its Estimation. *Archives of Toxicology* 56:215-218 (1985).

(4) Organization for Economic Cooperation and Development. OECD Guidelines for the Testing of Chemicals. OECD Guideline 425: Acute Oral Toxicity: Up-and-Down Procedure. Approved: June 1998.

(5) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 420: Acute Oral Toxicity—Fixed Dose Method, Adopted: July 17, 1992.

(6) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 423: Acute Oral Toxicity—Acute Toxic Class Method, Adopted: March 22, 1996.

(7) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 401: Acute Oral Toxicity, Adopted: February 24, 1987.

[65 FR 78771, Dec. 15, 2000]

§799.9120 TSCA acute dermal toxicity.

(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 toxic characteristics

of a substance, determination of acute dermal toxicity is useful where exposure by the dermal route is likely. It provides information on health hazards likely to arise from short-term exposure by the dermal route. Data from an acute study may serve as a basis for classification and labeling. It is an initial step in establishing a dosage regimen in subchronic and other studies and may provide information on dermal absorption and the mode of toxic action of a substance by this route. An evaluation of acute toxicity data should include the relationship, if any, between the exposure of animals 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 Office of Prevention, Pesticides, and Toxic Substances (OPPTS) harmonized test guideline 870.1200 (August 1998, final guideline). This source is available at the address in paragraph (f) of this section.

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

Acute dermal toxicity is the adverse effects occurring within a short time of dermal application of a single dose of a substance or multiple doses given within a 24-hour period.

Dosage is a general term comprising the dose, its frequency and the duration of dosing.

Dose is the amount of test substance applied. Dose is expressed as weight of test substance (grams, milligrams) per unit weight of test animal (e.g., milligrams per kilogram).

Dose-effect is the relationship between the dose and the magnitude of a defined biological effect either in an individual or in a population sample.

Dose-response is the relationship between the dose and the proportion of a population sample showing a defined effect.

LD₅₀ (median lethal dose), dermal, is a statistically derived estimate of a single dose of a substance that can be expected to cause death in 50% of treated animals when applied to the

skin. The LD₅₀ value is expressed in terms of weight of test substance per unit weight of test animal (milligrams per kilogram).

(d) *Approaches to the determination of acute toxicity.* (1) EPA recommends the following means to reduce the number of animals used to evaluate acute effects of chemical exposure while preserving 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 Agency encourages the review of existing acute toxicity information on mixtures that are substantially similar to the mixture under investigation. In certain cases it may be possible to glean enough information to make preliminary hazard evaluations that may reduce the need for further animal testing.

(ii) *Limit test.* When data on structurally related chemicals are inadequate, a limit test may be considered. If rodents are used, a limit dose of at least 2,000 mg/kg bodyweight may be administered to a single group of five males and five females using the procedures described in paragraph (e) of this section. If no lethality is demonstrated, no further testing for acute dermal toxicity is needed. If compound-related mortality is produced, further study may need to be considered.

(2) [Reserved]

(e) *Conventional acute toxicity test—(1) Principle of the test method.* The test substance is applied dermally in graduated doses to several groups of experimental animals, one dose being used per group. The doses chosen may be based on the results of a range finding test. Subsequently, observations of effects and deaths are made. Animals that die during the test are necropsied, and at the conclusion of the test the surviving animals are sacrificed and necropsied. This section is directed primarily to studies in either rats, rabbits, or guinea pigs but may be adapted for studies in other species. Animals showing severe and enduring signs of distress and pain may need to be humanely 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 in 40 CFR Part 792—Good Laboratory Practice Standards.

(3) *Test procedures*—(i) *Preparations.* 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 treatment groups.

(ii) *Animal selection*—(A) *Species and strain.* The rat, rabbit, or guinea pig may be used. The albino rabbit is preferred because of its size, ease of handling, skin permeability, and extensive data base. Commonly used laboratory strains must be employed. If a species other than rats, rabbits, or guinea pigs is used, the tester must provide justification and reasoning for its selection.

(B) *Age.* Young adult animals, rats between 8- and 12-weeks-old, rabbits at least 12-weeks-old, and guinea pigs between 5- and 6-weeks-old at the beginning of dosing should be used. The weight variation of animals used in a test must be within 20% of the mean weight for each sex.

(C) *Number and sex of animals.* (1) At least five experimentally naive animals with healthy intact skin are used at each dose level. They should all be of the same sex. After completion of the study in one sex, at least one group of five animals of the other sex is dosed 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 may be dispensed with. 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) The females must be nulliparous and nonpregnant.

(3) In acute toxicity tests with animals of a higher order than those mentioned above, the use of smaller numbers should be considered.

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

(E) *Housing.* Animals should be housed in individual cages.

(1) The temperature of the experimental animal rooms should be at 22 ± 3 °C for rodents, 20 ± 3 °C for rabbits.

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

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

(4) For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

(iii) *Dose levels and dose selection.* (A) Three dose levels must be used and spaced appropriately to produce test groups with a range of toxic effects and mortality rates. The data must be sufficient to produce a dose-response curve and permit an acceptable estimation of the median lethal dose. Range finding studies using single animals may help to estimate the positioning of the dose groups so that no more than three dose levels will be necessary.

(B) *Limit test.* This test is described in paragraph (d)(2)(ii) of this section.

(C) *Vehicle.* Solids should be pulverized when possible. The test substance should be moistened sufficiently with water or, where necessary, a suitable vehicle to ensure good contact with skin. If a vehicle or diluent is needed, it should not elicit toxic effects itself nor substantially alter the chemical or toxicological properties of the test substance. In addition, the influence of the vehicle on penetration of skin by the test substance should be taken into account. It is recommended that wherever possible the use of an aqueous solution be considered first, followed by consideration of a solution in oil (e.g., corn oil), and then by consideration of possible solution in other vehicles. For nonaqueous vehicles the toxic characteristics of the vehicle should be known, and if not known should be determined before the test. Acceptable alternative vehicles include gum arabic, ethanol and water, carboxymethyl cellulose, glycerol, propylene glycol,

PEG vegetable oil, and mineral oil as long as the vehicle is not irritating and the inability to use water or saline is justified in the report.

(iv) *Exposure and exposure duration.* The test substance must be administered over a period of 24 hours.

(v) *Preparation of animal skin.* Fur must be clipped from the dorsal area of the trunk of the test animals. Shaving may be employed, but it should be carried out at least 24 hours before dosing. Care must be taken to avoid abrading the skin, which would alter its permeability.

(vi) *Application of test substance.* (A) The test substance must be applied uniformly over a shaved or clipped area which is approximately 10% of the body surface area. The area starting at the scapulae (shoulders) to the wing of the ileum (hip bone) and half way down the flank on each side of the animal should be shaved or clipped. Liquid test materials should be undiluted if possible. 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. The test material is not removed until 24 hours after application. In the case where less than 10% of the surface area is covered an approximation of the exposed areas should be determined.

(B) The test substance must be held in contact with the skin with a porous gauze dressing (<8 ply) and nonirritating tape throughout a 24-hour exposure period. The test site must be further covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance. Restrainters may be used to prevent the ingestion of the test substance, but complete immobilization is not a recommended method. Although a semiocclusive dressing is preferred, an occlusive dressing will also be acceptable.

(C) At the end of the exposure period, residual test substance should be removed where practicable using water or an appropriate solvent.

(vii) *Observation period.* Although 14 days is recommended as a minimum observation period, 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 may thus be extended when considered necessary. The time at which signs of toxicity appear, their duration, and the time to death are important, especially if there is a tendency for deaths to be delayed.

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

(B) Additional observations must be made daily, especially in the early days of the study. 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 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 shortly before the test substance is administered, weekly thereafter, and at death. Changes in weights should be calculated and recorded when survival exceeds one day.

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

(ix) *Gross pathology.* (A) At the end of the test, surviving animals must be weighed and sacrificed.

(B) A gross necropsy must be performed on all animals under test. All gross pathology changes should be recorded.

(C) If necropsy cannot be performed immediately after a dead animal is discovered, the animal should be refrigerated (not frozen) at temperatures low enough to minimize autolysis. Necropsies should be performed as soon as practicable, normally within a day or two.

(x) *Additional evaluations.* Microscopic examination of organs showing

evidence of gross pathology in animals surviving 24 hours or more should also be considered because it may yield useful information.

(xi) *Data and reporting*—(A) *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 dose levels, number of animals displaying other signs of toxicity, description of toxic effects and necropsy findings. Any methods used for calculation of the LD₅₀ or any other parameters should be specified and referenced. Methods for parameter estimation are described in the references listed in paragraphs (f)(1), (f)(2), and (f)(3) of this section.

(B) *Evaluation of results*. An evaluation should include the relationship, if any, between exposure of the animals to the test substance and the incidence and severity of all abnormalities, including behavioral and clinical abnormalities, gross lesions, body weight changes, effects on mortality, and any other toxic effects. The LD₅₀ value should always be considered in conjunction with the observed toxic effects and any necropsy findings. The LD₅₀ value is a relatively coarse measurement, useful only as a reference value for classification and labeling purposes, and for an expression of the lethal potential of the test substance by the dermal route. Reference should always be made to the experimental animal species in which the LD₅₀ value was obtained.

(C) *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 must include:

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

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

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

(4) Tabulation of individual and test group data by sex and dose 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).

(i) Description of toxic effects, including their time of onset, duration, reversibility, and relationship to dose.

(ii) Body weights.

(iii) Time of dosing and time of death after dosing.

(iv) Dose-response curves for mortality and other toxic effects (when permitted by the method of determination).

(v) Gross pathology findings.

(vi) Histopathology findings and any additional clinical chemistry evaluations, if performed.

(5) Description of any pre-test conditioning, including diet, quarantine and treatment for disease.

(6) 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.

(7) Manufacturer, source, purity, and lot number of test substance.

(8) Relevant properties of substance tested including physical state and pH (if applicable).

(9) Identification and composition of any vehicles (*e.g.*, diluents, suspending agents, and emulsifiers) or other materials used in administering the test substance.

(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.

(1) Chanter, D.O. and Heywood, R., The LD₅₀ Test: Some Considerations of Precision, *Toxicology Letters* 10:303-307 (1982).

(2) Finney, D.J. Chapter 3—Estimation of the median effective dose and Chapter 4—Maximum likelihood estimation, *Probit Analysis*, 3rd ed. Cambridge, London (1971).

(3) Finney, D.J. The Median Lethal Dose and Its Estimation. *Archives of Toxicology* 56:215-218 (1985).

(4) Organization for Economic Cooperation and Development. OECD Guideline for the Testing of Chemicals. OECD Guideline 425: Acute Oral Toxicity: Up-and-Down Procedure. Adopted: September 21, 1998.

(5) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 420: Acute Oral Toxicity—Fixed Dose Method. Adopted: July 17, 1992.

(6) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 423: Acute Oral Toxicity—Acute Toxic Class Method. Adopted: March 22, 1996

(7) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 402: Acute Dermal Toxicity. Adopted: February 24, 1987.

[65 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 aerodynamic diameter (MMAD) and either 84% or 16% of the diameter size distribution (e.g., MMAD = 2 μ m; 84% = 4 μ m; 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 aerodynamic diameter of a particle which is considered to be inhalable for the organism 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 exposure or within a fixed time after exposure in 50% of animals exposed for a specified time. The LC₅₀ value is a relatively coarse measurement useful only for classification and labeling purposes and an expression of the lethal potential of the test substance following inhalation. The LC₅₀ value is expressed as weight of test substance per unit volume of air (milligrams per