(D) The extracting solvent used, and its extraction efficiency.

(E) The average and standard deviation of solute concentration in each collection vessel.

(F) Any changes made or problems encountered in the test procedure.

(G) If applicable, a complete description of the analytical method which was used instead of the GC method.

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

(1) DeVoe, H. et al., Generator columns and high pressure liquid chromatography for determining aqueous solubilities and octanol-water partition coefficients of hydrophobic substances. *Journal of Research, National Bureau of Standards,* 86:361–366 (1981).

(2) Hansch, C. et al., The linear free-energy relationship between partition coefficients, and the aqueous solubility of organic liquids. *Journal of Organic Chemistry* 33:347-350 (1968).

(3) Leifer, A. et al., Environmental transport and transformation of polychlorinated biphenyls. Chapter 1. U.S. Environmental Protection Agency Report: EPA-560/5–83–005 (1983).

(4) Mackay, D. et al., Relationships between aqueous solubility and octanol-water partition coefficient. *Chemosphere* 9:701-711 (1980).

(5) May, W.E. et al., Determination of the aqueous solubility of polynuclear aromatic hydrocarbons by a coupled column liquid chromatographic technique. *Analytical Chemistry* 50:175–179 (1978).

(6) May, W.E. et al. Determination of the solubility behavior of some polycyclic aromatic hydrocarbons in the water. *Analytical Chemistry,* 50:997–1000 (1978a).

(7) Miller, N.M. et al., Aqueous solubilities, octanol/water partition coefficients, and entropy of melting of chlorinated benzenes and biphenyls. *Journal of Chemical and Engineering Data* 29:184-190 (1984).

(8) OECD/Organization for Economic Cooperation and Development. Test Guideline No. 105. Water solubility column elution-flask method (1981).

(9) Sutton, C. and Calder, J.A., Solubility of alkylbenzenes in distilled water and seawater at 25 °C. *Journal of Chemical and Engineering Data* 20:320–322 (1975).

(10) Tewari, Y.B. et al., Aqueous solubility and octanol/water partition coefficient of organic compounds at 25 °C. *Journal of Chemical and Engineering Data* 27:451-454 (1982).

(11) Wasik, S.P. et al., Octanol/Water Partition Coefficient and Aqueous Solubilities of Organic Compounds. NBS Report NBSIR 81–2406. Washington, DC: National Bureau of Standards, U.S. Department of Commerce (1981).

(12) Yalkowski, S.H. et al., ''Aquasol database of aqueous solubilities of organic compounds''; Fifth Edition. University of Arizona, College of Pharmacy, Tucson, AZ 85721 (1990) (available at *http://www.pharm.arizona.edu/aquasol/index.html*).

(13) ASTM D 1193–91, *Standard Specification for Reagent Water.* American Society for Testing and Materials (ASTM). 1916 Race St., Philadelphia, PA 19103.

Subparts F–G [Reserved]

Subpart H—Health Effects Test Guidelines

SOURCE: 62 FR 43824, Aug. 15, 1997, unless otherwise noted.

§ 799.9110 TSCA acute oral 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 oral toxicity is usually an initial step. It provides information on health hazards likely to arise from short-term exposure by the oral 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 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 870.1100 (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 oral toxicity is the adverse effects occurring within a short period of time after oral administration of either 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 administered. Dose is expressed as weight of test substance (milligrams, grams) 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.

LD_{50} (median lethal dose) is a statistically derived estimate of single dose of a substance that can be expected to cause death in 50% of animals when administered by the oral route. The LD_{50} value is expressed in terms of weight of test substance per unit weight of test animal (milligrams per kilogram).

(d) *Alternative approaches to the determination of acute toxicity.* (1) EPA will accept the following procedures to reduce the number of animals used to evaluate acute effects of chemical exposure while preserving its ability to make reasoned judgments about safety:

(i) *Estimation of acute oral toxicity.* When further study is warranted, EPA generally supports limiting such tests to those using the lowest number of animals feasible. EPA will accept three alternative Organization for Economic Cooperation and Development (OECD) test methods in place of the ''traditional'' acute oral toxicity test. The three OECD alternatives are the following:

(A) The up and down procedure as described in OECD Guideline 425 referenced in paragraph (f)(4) of this section.

(B) The acute toxic class method as described in OECD Guideline 423 and referenced in paragraph (f)(6) of this section.

(C) The fixed dose method as described in OECD Guideline 420 and referenced in paragraph (f)(5) of this section.

(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 per kilogram of body weight 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 oral toxicity is needed. (Under current policy and regulations for pesticide products, precautionary statements may still be required unless there are data to indicate the LD_{50} is greater than 5,000 mg/kg.) If compound-related mortality is produced in the limit test, further study may need to be considered.

(2) [Reserved]

(e) *Conventional acute toxicity test*—(1) *Principle of the test method.* The test substance is administered orally by gavage 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 rodent species but may be adapted for studies in nonrodents. 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 described 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.* Although several mammalian test species may be used, the rat is the preferred species. Commonly used laboratory strains must be employed. If another species is used, the tester must provide justification and reasoning for its selection.

(B) *Age.* Young adult rats between 8- and 12-weeks-old at the beginning of dosing should be used. Rabbits should be at least 12 weeks of age at study initiation. 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 rodents 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 rodents, the use of smaller numbers should be considered.

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

(E) *Housing.* 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.

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

(*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, spaced appropriately to produce test groups with a range of toxic effects and mortality rates. The data collected must be sufficient to produce a dose-response curve and permit an acceptable estimation of the LD_{50}. Range finding studies using single animals may help to estimate the positioning of dose groups so that no more than three dose levels will be necessary.

(B) *Limit test.* This test has been defined and described in paragraph (d)(1)(ii) of this section.

(C) *Vehicle.* 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 nor substantially alter the chemical or toxicological properties of the test substance. 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. Toxic characteristics of non-aqueous vehicles should be known, and, if not known, should be determined before the test.

(D) *Volume.* The maximum volume of liquid that can be administered at one time depends on the size of the test

animal. In rodents, the volume should not exceed 1 mL/100 g body weight, except when an aqueous solution is used in which case 2 mL/100 g may be administered. Either constant volume or constant concentration administration is acceptable when dosing, provided the following guidance is employed. When possible, the liquid test material should be dosed neat. Otherwise, it may be diluted, using the highest concentration possible, although volumes less than 0.5 mL per animal would not be required. Lower dose volumes are acceptable if they can be accurately administered. Solid materials should be suspended or dissolved in the minimum amount of vehicle and dosed at the highest concentration possible.

(iv) *Exposure and exposure duration.* (A) Animals must be fasted prior to test substance administration. For the rat, feed should be withheld overnight; for other rodents with higher metabolic rates a shorter period of fasting is appropriate.

(B) The test substance must be administered in a single dose by gavage, using a stomach tube or suitable intubation cannula.

(C) If a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours. Where a dose is administered in fractions, it may be necessary to provide the animals with food and water, depending on the length of the dosing period.

(D) After the substance has been administered, feed may be withheld for an additional 3–4 hours.

(v) *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.

(vi) *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 1 day.

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

(vii) *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.

(viii) *Additional evaluation.* 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.

(ix) *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 LD_{50} 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 LD_{50} value should always be considered in conjunction with the observed toxic effects and any necropsy findings. The LD_{50} 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 LD_{50} 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 Nonconfidential 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 LD_{50} 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.

LD_{50} (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 LD_{50} 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,