(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 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 L_{D50} test: some considerations of precision. *Toxicology Letters* 10:303 307 (1982).

(2) Finney, D.G. Chapter 3 Estimation of the median effective dose, 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. Final Draft OECD Guideline 425: Acute Oral Toxicity: Up-and-Down Procedure to be adopted in the Tenth Addendum to the OECD Guidelines for the Testing of Chemicals.

(5) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 403: Acute Inhalation Toxicity. Adopted: May 12, 1981.

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

(7) 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.

(8) U. S. EPA. Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies. 2/1/94. Health Effects Division, Office of Pesticide Programs.

[65 FR 78776, Dec. 15, 2000]

§ 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 latter is of particular concern in relation to spills and other accidental releases. This testing is designed to determine the gross pathology and histopathology resulting from acute inhalation exposure to a substance. Because toxic effects on the respiratory tract are of particular concern following inhalation exposure, several indicators of respiratory toxicity consisting of histopathology on fixed tissue and evaluation of cellular and biochemical parameters in bronchoalveolar lavage fluid should be employed. The respiratory histopathology consists of specialized techniques to preserve tissues of the respiratory tract in order to allow detailed microscopic examination to identify adverse effects of chemical substances on this organ system. The bronchoalveolar lavage is designed to be a rapid screening test to provide an early indicator of pulmonary toxicity by examining biochemical and cytologic endpoints of material from the lungs of animals exposed to potentially toxic chemical substances. These acute tests are designed to assess the relationship, if any, between the animals' exposure to the test substance and to demonstrate relationship between the animals' exposure and the incidence and severity of observed abnormalities, including gross or histopathologic lesions, body weight changes, effects on mortality, and any other toxic effects. These acute tests are not intended to provide a complete evaluation of the toxicologic effects of a substance, and additional functional and morphological evaluations may be necessary to assess completely the potential effects produced by a chemical

substance. Additional tests may include longer-term exposures, or more in-depth evaluation of specific organ systems as indicated by signs of toxicity following acute exposure.

(b) *Source.* This a new section developed by the United States Environmental Protection Agency.

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

Aerodynamic diameter (d_{ae}) refers to the size of particles. It is the diameter of a sphere of unit density that behaves aerodynamically (has the same settling velocity in air) as the particle of the test substance. It is used to compare particles of different size, shape, and density, and to predict where in the respiratory tract such particles may be primarily deposited.

Exposure response is the relationship between the exposure concentration and the measured toxic response, whether expressed as a group mean ± standard deviation) in the case of a continuous variable or as incidence in the case of a quantal variable. This definiton should not preclude the exploration of other dose metrics in establishing this relationship.

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 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).

Inhalability is the ratio of the number concentration of particles of a certain aerodynamic diameter, d_{ae}, that are inspired through the nose or mouth to the number concentration of the same d_{ae} present in the inspired volume of ambient air. In humans, inhalability can exceed 15 μm d_{ae}, whereas inhalability dramatically decreases for particles above 4 μm d_{ae} in small laboratory animals.

Lower respiratory tract consists of those structures of the respiratory tract below the larynx.

Mass geometric mean aerodynamic diameter or the mass median aerodynamic diameter (MMAD) is the calculated aerodynamic diameter that divides the particles of an aerosol (a gaseous suspension of fine liquid or solid particles) in half, based on the weight of the particles. By weight, 50% of the particles will be larger than the MMAD and 50% of the particles will be smaller than the MMAD.

Particle regional deposition is the fraction of inhaled particles that deposits in the specific region of the respiratory tract. The major mechanisms of particle deposition in the respiratory tract include impaction, sedimentation, diffusion, interception, and electrostatic precipitation. The deposition mechanism that is dominant for a given region depends on the respiratory tract architecture and ventilation rate of the species and the aerosol particle size and distribution. The respiratory tract in both humans and various experimental mammals can be divided into three regions on the basis of structure, size, and function:

(1) The extrathoracic region or upper respiratory tract that includes the nose, mouth, nasopharynx, oropharynx, laryngopharynx, and larynx.

(2) The tracheobronchial region that includes the trachea, bronchi, and bronchioles (including the terminal bronchioles).

(3) The alveolar region that includes the respiratory bronchioles (if present in the species), alveolar ducts, alveolar sacs, and alveoli.

Respiratory effects are any adverse effects on the structure or functions of the respiratory system related to exposure to a chemical substance.

Target organ is any organ found to be a target of toxicity in the 4-hour (hr) high concentration group as a result of:

(1) The initial histopathologic examination (respiratory tract, liver, kidney, gross lesions); or

(2) The retrospective histopathologic examination of archived organs triggered by their identification as targets of toxicity in a 90-day study.

Toxic effects are any adverse changes (a change that is statistically and biologically significant) in the structure or function of an experimental animal as a result of exposure to a chemical substance.

Upper respiratory tract consists of those structures of the respiratory tract above and including the larynx.

(d) *Principle of the test method.* The test substance shall be administered to several groups of experimental animals; one concentration level and duration being used per group. Bronchoalveolar lavage shall be used to evaluate early effects on the respiratory system by examining changes in the content of the lavage fluid of the lung. At 24 hrs following exposure, the animals shall be sacrificed and necropsied, and tissue samples from the respiratory tract and other major organs will be prepared for microscopic examination. The exposure levels at which significant toxic effects on the respiratory organ system are produced are compared to those levels that produce other toxic effects. As triggered by the results of the 4-hr test, additional exposure periods of 1 hr and 8 hrs will be required to determine the effect of exposure time on the toxicity observed. A 1-hr exposure study can be elected as an option to provide data suitable for risk assessment for very short duration exposures as may occur from chemical releases. In the absence of adequate toxicological data for 1-hr exposure, the Agency will extrapolate to shorter-term exposures from the 4-hr data on the basis of concentration alone. This is a conservative method of extrapolation, consistent with general Agency methods for deriving criteria for short-term exposure from longer-term studies (a concentration x time extrapolation would result in higher concentration for a shorter duration).

(e) *Test procedures*—(1) *Animal selection*—(i) *Species.* In general, the laboratory rat and mouse should be used. Under some circumstances, other species, such as the hamster or guinea pig, may be more appropriate, and if these or other species are used, justification should be provided.

(ii) *Strain.* If rats and mice are used, the use of the F344 rat and the B6C3F1 mouse is preferred to facilitate comparison with existing data.

(iii) *Age.* Young adults shall be used. The weight variation of animals used in a test should not exceed ' 61' 20% of the mean weight for each species.

(iv) *Sex.* Equal numbers of animals of each sex shall be used for each concentration level. The females shall be nulliparous and nonpregnant.

(v) *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 shall be monitored for known viral and bacterial respiratory pathogens determined by conventional microbiological assays (e.g., serology). The animals shall be free from pathogens at the start of exposure.

(2) *Number of animals.* At least five males and five females shall be used in each concentration/duration and control group. Animals shall be randomly assigned to treatment and control groups.

(3) *Control groups.* The control group shall be a sham-treated 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 is used to help generate an appropriate concentration of the substance in the atmosphere, a vehicle control group shall be used. If the 4- and 8-hr exposure studies are conducted concurrently, a concurrent 8-hr sham-exposed control group may serve as the control group for both the 4-hr and the 8-hr exposure studies, provided there is adequate historical control data showing no changes in histopathology or bronchoalveolar lavage of controls exposed for 4 and 8 hrs. Similarly, if the optional 1-hr exposure study is conducted concurrently with the 4- and/or 8-hr study, the concurrent control group for those studies may also be used for the 1-hr study, provided adequate historical control data show no changes in histopathology or bronchoalveolar lavage between controls exposed for these time periods.

(4) *Concentration level and concentration selection.* For the 4-hr study, at least three concentrations shall be used in addition to the control group. Ideally, the data generated from the test should be sufficient to produce an exposure-response curve. 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-adverse-effects level (NOAEL).

(i) *Limit concentration.* For aerosols and particles, the high concentrations need not be greater than 2 mg/L, or concentrations that cannot maintain a particle size distribution having an MMAD between 1 and 4 μm (i.e., a particle size that permits inhalability and deposition throughout the respiratory tract). 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 particulate exposure of 2 mg/L (actual concentration of respirable substance) for 4 hrs or, where this is not feasible, the maximum attainable concentration, using the procedures described for this study, produces no observable toxic effects, then a full study using three concentrations will not be necessary. 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 effects, then a full study using three concentrations will not be necessary.

(ii) *8-hr study and optional 1-hr study.* If the 8-hr study is triggered, three concentrations shall be tested. These concentrations should allow for the determination of an effect level and a NOAEL. If the option to perform a 1-hr study is elected, three concentrations shall be selected and tested in a similar manner.

(5) *Inhalation exposure.* Animals can be exposed to the substance by either a nose-only procedure or in a whole-body exposure chamber.

(i) *Inhalation chambers.* The animals shall 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 hr 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 shall 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.

(ii) *Environmental conditions.* The temperature at which the test is performed shall 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.

(iii) *Exposure periodicity.* For acute testing, the exposure design shall enable 4 hrs of exposure to the target concentrations, as defined by an average of ± 5% for gases and vapors and ± 15% for particles and aerosols. If triggered by the results of the 4-hr exposure, additional testing shall be conducted in a comparable manner using an 8-hr exposure period.

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

(i) Chemical purity of the test material shall be analyzed.

(ii) The rate of airflow shall be monitored continuously, but shall be recorded at least every 30 minutes.

(iii) The actual concentrations of the test substance shall be measured in the breathing zone. During the exposure period, the actual concentrations of the test substance shall be held as constant as practical, monitored continuously or intermittently depending on the method of analysis, and recorded at least at the beginning, at an intermediate time, and at the end of the exposure period. Well-established and published monitoring methods should be used where available. If no standard methods are available, then accuracy and precision information must be supplied.

(iv) During the development of the generating system, appropriate particle size analysis shall be performed to

establish the stability of the aerosol. During exposure, analysis should be conducted as often as necessary to determine the consistency of particle size distribution. The particle size distribution shall have an MMAD between 1 and 4 μm. The particle size of hygroscopic materials shall be small enough when dry to assure that the size of the particle at saturation will still have an MMAD between 1 and 4 μm. Characterization for fibers shall include the bivariate distribution of length and diameter; this distribution must ensure inhalability.

(v) If the test substance is present in a mixture, the mass and composition of the entire mixture, as well as the principal compound, shall be measured.

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

(7) *Food and water during exposure period.* Food shall be withheld during exposure. Water may also be withheld in certain cases.

(8) *Observation period.* The bronchoalveolar lavage and respiratory pathology shall be conducted 24 hrs following exposure to allow expression of signs of toxicity. There is concern that some latency time will be required to allow migration of cells and macromolecules into the lungs following exposure, and that some pathology may require macromolecular synthesis or degradation before cell damage develops.

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

(ii) At least the lungs, liver, kidneys, adrenals, brain, and gonads shall be 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: 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; liver; spleen; kidneys; adrenals; pancreas; gonads; accessory genital organs (epididymis, prostrate, 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 shall also be preserved in a suitable medium.

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

(i) Full histopathology shall be performed on the respiratory tract, liver and kidney of all animals in the control and high concentration groups. The histopathology of the respiratory tract is described under paragraph (e)(11) of this section.

(ii) All gross lesions which differ from controls in frequency, distribution, type, or severity in all concentration groups.

(iii) Target organs in all animals, as indicated by the observations in the high concentration group in this study. Histopathologic examination of target organs in animals at all concentration levels (rather than only to the extent necessary to define the NOAEL) can support the application of exposure-response analyses such as the benchmark concentration approach.

(iv) Archived organs identified as targets of toxicity from results of the 90-day study (if a 90-day study is required for this substance) should be elevated in high concentration animals of the 4-hr acute study to determine if they are also targets of acute toxicity.

(11) *Respiratory tract histopathology.* (i) Representative sections of the respiratory tract shall be examined histologically. These shall include the trachea, major conducting airways, alveolar region, terminal and respiratory bronchioles (if present), alveolar ducts and sacs, and interstitial tissues.

(ii) Care shall be taken that the method used to kill the animal does not result in damage to the tissues of the upper or lower respiratory tract. The lungs shall be infused with a fixative while in an inflated state of fixed pressure.

(iii) The upper respiratory tract shall be examined for histopathologic lesions. This examination shall use a minimum of four sections located as

specified under paragraphs (e)(11)(iii)(A) through (e)(11)(iii)(D) of this section. An evaluation of the nasal vestibule shall be conducted. The method described by the reference under paragraph (h)(11) of this section should be given consideration. The use of additional sections shall be left to the discretion of the study pathologist, but consideration should be given to additional sections as recommended in the reference under paragraph (h)(8) of this section to ensure adequate evaluation of the entire upper respiratory tract, particularly the nasopharyngeal meatus. The following transverse sections shall be examined:

(A) Immediately posterior to the upper incisor teeth.

(B) At the incisor papilla.

(C) At the second palatal ridge.

(D) At the level of the first upper molar teeth.

(iv) The laryngeal mucosa shall be examined for histopathologic changes. Sections of the larynx to be examined include the epithelium covering the base of the epiglottis, the ventral pouch, and the medial surfaces of the vocal processes of the arytenoid cartilages.

(12) *Bronchoalveolar lavage.* (i) Animals can be exposed to the substance by either a nose-only procedure or in a whole-body exposure chamber.

(ii) Care should be taken that the method used to kill the animal results in minimum changes in the fluid of the lungs of the test animals.

(iii) At the appropriate time, the test animals shall be killed and the heart-lung including trachea removed in bloc. Alternatively, lungs can be lavaged in situ. If the study will not be compromised, one lobe of the lungs may be used for lung lavage while the other is fixed for histologic evaluation. The lungs should be lavaged using physiological saline. The lavages shall consist of two washes, each of which consists of approximately 80% (e.g., 5 ml in rats and 1 ml in mice) of the total lung volume. Additional washes merely tend to reduce the concentrations of the material collected. The lung lavage fluid shall be stored on ice at 5 °C until assayed.

(iv) The following parameters shall be determined in the lavage fluid as indicators of cellular damage in the lungs: total protein, cell count, and percent leukocytes. In addition, a phagocytosis assay shall be performed to determine macrophage activity. Assay methods described in the references under paragraphs (h)(1) and (h)(3) of this section may be used.

(13) *Combined protocol.* The tests described may be combined with any other toxicity study, as long as none of the requirements of either are violated by the combination.

(f) *Triggered testing.* If no adverse effects are seen in the 4-hr study as compared with controls, no further testing is necessary. If the 4-hr study shows positive effects in histopathology or the bronchoalveolar lavage, an 8-hr study shall be conducted. Only those tissues showing positive results in the 4-hr study must be pursued in the follow-up 8-hr study. Similarly, if the option to perform a 1-hr study is exercised, only those tissues showing positive results in the 4-hr study shall be pursued.

(g) *Data reporting and evaluation.* The final test report shall include the following information:

(1) *Description of equipment and test methods.* A description of the general design of the experiment and any equipment used shall be provided.

(i) Description of exposure apparatus, including design, type, dimensions, source of air, system for generating particles, aerosols, gasses, and vapors, method of conditioning air, treatment of exhaust air, and the method of housing animals in a test chamber.

(ii) Description of the equipment for measuring temperature, humidity, and particulate aerosol concentration and size.

(iii) Exposure data shall be tabulated and presented with mean values and 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 air.

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

(E) Actual concentration in test breathing zone.

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

(2) *Results*—(i) *General group animal data*. The following information shall be arranged by test group exposure level.

(A) Number of animals exposed.

(B) Number of animals dying.

(C) Number of animals showing overt signs of toxicity.

(D) Pre- and post-exposure body weight change in animals, and weight change during the observation period.

(ii) *Counts and incidence of gross alterations observed at necropsy in the test and control groups*. Data shall be tabulated to show:

(A) The number of animals used in each group and the number of animals in which any gross lesions were found.

(B) The number of animals affected by each different type of lesion, and the locations and frequency of each type of lesion.

(iii) *Counts and incidence of general histologic alterations in the test group*. Data shall be tabulated to show:

(A) The number of animals used in each group and the number of animals in which any histopathologic lesions were found.

(B) The number of animals affected by each different type of lesion, and the locations, frequency, and average grade of each type of lesion.

(iv) *Counts and incidence of respiratory histopathologic alterations by the test group*. Data shall be tabulated to show:

(A) The number of animals used in each group and the number of animals in which any histopathologic lesions were found.

(B) The number of animals affected by each different type of lesion, and the locations, frequency, and average grade of each type of lesion.

(v) *Results of the bronchoalveolar lavage study*. Data shall be tabulated to show:

(A) The amount of administered lavage fluid and recovered lavage fluid for each test animal.

(B) The magnitude of change of biochemical and cytologic indices in lavage fluids at each test concentration for each animal.

(C) Results shall be quantified as amount of constituent/mL of lavage fluid. This assumes that the amount of lavage fluid recovered is a representative sample of the total lavage fluid.

(3) *Evaluation of data*. The findings from this acute study should be evaluated in the context of preceding and/or concurrent toxicity studies and any correlated functional findings. The evaluation shall include the relationship between the concentrations of the test substance and the presence or absence, incidence, and severity of any effects. The evaluation should include appropriate statistical analyses, for example, parametric tests for continuous data and non-parametric tests for the remainder. Choice of analyses should consider tests appropriate to the experimental design, including repeated measures. The report must include concentration-response curves for the bronchoalveolar lavage and tables reporting observations at each concentration level for necropsy findings and gross, general, and respiratory system histopathology.

(h) *Reference*. 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., SW., Washington, DC, 12 noon to 4 p.m., Monday through Friday, except legal holidays.

(1) Burleson, G.R., Fuller, L.B.;e nache, M.G., and Graham, J.A. Poly (I): poly (C)-enhanced alveolar peritoneal macrophage phagocytosis: Quantification by a new method utilizing fluorescent beads. *Proceedings of the Society of Experimental Biology and Medicine*. 184:468–476 (1987).

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(10) Renne, R.A., Gideon, K.M., Miller, R.A., Mellick, P.W., and Grumbein, S.L. Histologic methods and interspecies variations in the laryngeal histology of F344/N rats and B6C3F1 mice. *Toxicology and Pathology*. 20:44–51 (1992).

(11) Young, J.T. Histopathologic examination of the rat nasal cavity. *Fundamental and Applied Toxicology*. 1:309–312 (1981).

§ 799.9305 TSCA Repeated dose 28–day oral toxicity study in rodents.

(a) *Scope*—(1) *Applicability*. This section is intended to meet testing requirements of the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) *Source*. The source material used in developing this TSCA test guideline is the Office of Prevention, Pesticides and Toxic Substances (OPPTS) harmonized test guideline 870.3050 (July 2000, final guidelines). This source is available at the address in paragraph (h) of this section.

(b) *Purpose*. (1) In the assessment and evaluation of the toxic characteristics of a chemical, the determination of oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained by acute testing. This study provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time. The method comprises the basic repeated dose toxicity study that may be used for chemicals on which a 90–day study is not warranted (*e.g.*, when the production volume does not exceed certain limits) or as a preliminary to a long term study. The duration of exposure should normally be 28 days although a 14–day study may be appropriate in certain circumstances; justification for use of a 14–day exposure period should be provided.

(2) This section places emphasis on neurological effects as a specific endpoint, and the need for careful clinical observations of the animals, so as to obtain as much information as possible, is stressed. The method should identify chemicals with neurotoxic potential, which may warrant further in-depth investigation of this aspect. In addition, the method may give an indication of immunological effects and reproductive organ toxicity.

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

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

Dose is the amount of test substance administered. Dose is expressed as weight (g, mg) or as weight of test substance per unit weight of test animal (e.g., mg/kg), or as constant dietary concentrations (parts per million (ppm)).

No-observed-effects level (NOEL) is the maximum dose used in a study which produces no adverse effects. The NOEL is usually expressed in terms of the weight of a test substance given daily per unit weight of test animals (milligrams per kilograms per day).