

## Environmental Protection Agency

§ 799.9310

H.A., MacPhail R.C. (1991). Interlaboratory Comparison of Motor Activity Experiments: Implication for Neurotoxicological Assessments. *Neurotoxicology and Teratology*, 13:599-609.

[65 FR 78780, Dec. 15, 2000]

### § 799.9310 TSCA 90-day oral toxicity in rodents.

(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 chemical, the determination of subchronic oral toxicity may be carried out after initial information on toxicity has been obtained by acute testing. The subchronic oral study has been designed to permit the determination of the no-observed-effects level (NOEL) and toxic effects associated with continuous or repeated exposure to a test substance for a period of 90 days. This study is not capable of determining those effects that have a long latency period for development (e.g., carcinogenicity and life shortening). Extrapolation from the results of this study to humans is valid only to a limited degree. However, it can be useful in providing information on health hazards likely to arise from repeated exposure by the oral route over a limited period of time, such as target organs, the possibilities of accumulation, and can be of use in selecting dose levels for chronic studies and for establishing safety criteria for human exposure.

(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 870.3100 (August 1998, final guideline). This source is available at the address in paragraph (h) of this section.

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

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

*Dose* in a subchronic oral study is the amount of test substance administered

daily via the oral route (gavage, drinking water or diet) for a period of 90 days. Dose is expressed as weight of the test substance (grams, milligrams) per unit body weight of test animal (milligram per kilogram) or as weight of the test substance in parts per million in food or drinking water per day.

*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 animal (milligrams per kilogram per day).

*Subchronic oral toxicity* is the adverse effects occurring as a result of the repeated daily exposure of experimental animals to a chemical by the oral route for a part (approximately 10%) of the test animal's life span.

*Target organ* is any organ of a test animal showing evidence of an effect induced by a test substance.

(d) *Limit test*. If a test at one dose level of at least 1,000 mg/kg body weight (expected human exposure may indicate the need for a higher dose level), using the procedures described for this study, produces no observable toxic effects or if toxic effects would not be expected based upon data of structurally related compounds, then a full study using three dose levels might not be necessary.

(e) *Test procedures*—(1) *Animal selection*—(i) *Species and strain*. A variety of rodent species may be used, although the rat is the preferred species. Commonly used laboratory strains must be employed.

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

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

(C) At the commencement of the study the weight variation of animals used must be within 20% of the mean weight for each sex.

(iii) *Sex*. Equal numbers of animals of each sex must be used at each dose level, and the females shall be nulliparous and nonpregnant.

(iv) *Numbers*. (A) At least 20 rodents (10 males and 10 females) at each dose level.

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

(C) To avoid bias, the use of adequate randomization procedures for the proper allocation of animals to test and control groups is required.

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

(v) *Husbandry.* (A) Animals may be group-caged by sex, but the number of animals per cage must not interfere with clear observation of each animal. The biological properties of the test substance or toxic effects (e.g., morbidity, excitability) may indicate a need for individual caging.

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

(C) The relative humidity of the experimental animal rooms should be  $50 \pm 20\%$ .

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

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

(F) The study should not be initiated until animals have been allowed a period of acclimatization/quarantine to environmental conditions, nor should animals from outside sources be placed on test without an adequate period of quarantine. An acclimation period of at least five days is recommended.

(2) *Control and test substances.* (i) Where necessary, the test substance is dissolved or suspended in a suitable vehicle. If a vehicle or diluent is needed, the vehicle should not elicit toxic effects or substantially alter the chemical or toxicological properties of the test substance. It is recommended that wherever possible the usage of an aqueous solution be considered first, fol-

lowed by consideration of a solution in oil and then solution in other vehicles.

(ii) If possible, one lot of the test substance tested should be used throughout the duration of the study and the research sample should be stored under conditions that maintain its purity and stability. Prior to the initiation of the study, there should be a characterization of the test substance, including the purity of the test compound and, if technically feasible, the names and quantities of contaminants and impurities.

(iii) If the test or control substance is to be incorporated into feed or another vehicle, the period during which the test substance is stable in such a mixture should be determined prior to the initiation of the study. Its homogeneity and concentration should be determined prior to the initiation of the study and periodically during the study. Statistically randomized samples of the mixture should be analyzed to ensure that proper mixing, formulation, and storage procedures are being followed, and that the appropriate concentration of the test or control substance is contained in the mixture.

(3) *Control groups.* A concurrent control group is required. This group must be an untreated or sham-treated control group or, if a vehicle is used in administering the test substance, a vehicle control group. If the toxic properties of the vehicle are not known or cannot be made available, both untreated and vehicle control groups are required.

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

(5) *Dose levels and dose selection.* (i) In subchronic toxicity tests, it is desirable to determine a dose-response relationship as well as a NOEL. Therefore, at least three dose levels plus a control and, where appropriate, a vehicle control (corresponding to the concentration of vehicle at the highest dose

level) must be used. Doses should be spaced appropriately to produce test groups with a range of toxic effects. The data should be sufficient to produce a dose-response curve.

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

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

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

(6) *Administration of the test substance.*

(i) If the test substance is administered by gavage, the animals are dosed with the test substance on a 7-day per week basis for a period of at least 90 days. However, based primarily on practical considerations, dosing by gavage on a 5-day per week basis is acceptable. If the test substance is administered in the drinking water, or mixed in the diet, then exposure should be on a 7-day per week basis.

(ii) All animals must be dosed by the same method during the entire experimental period.

(iii) For substances of low toxicity, it is important to ensure that when administered in the diet the quantities of the test substance involved do not interfere with normal nutrition. When the test substance is administered in the diet, either a constant dietary concentration (parts per million) or a constant dose level in terms of body weight should be used; the alternative used should be specified.

(iv) For a substance administered by gavage, the dose should be given at approximately the same time each day, and adjusted at intervals (weekly or bi-weekly) to maintain a constant dose level in terms of body weight.

(7) *Observation period.* (i) The animals must be observed for a period of 90 days.

(ii) Animals in the satellite group (if used) scheduled for follow-up observations should be kept for at least 28 days further without treatment to detect recovery from, or persistence of, toxic effects.

(8) *Observation of animals.* (i) Observations must be made at least twice each day for morbidity and mortality. Appropriate actions should be taken to

minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals). General clinical observations should be made at least once a day, preferably at the same time each day, taking into consideration the peak period of anticipated effects after dosing. The clinical condition of the animal should be recorded.

(ii) A careful clinical examination must be made at least once weekly. Observations should 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 stereotypes or bizarre behavior (e.g., self-mutilation, walking backwards).

(iii) Signs of toxicity should be recorded as they are observed including the time of onset, degree and duration.

(iv) Measurements of food consumption and water consumption, if drinking water is the exposure route, must be made weekly.

(v) Individual weights of animals must be determined shortly before the test substance is administered, weekly thereafter, and at death.

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

(vii) At termination, all survivors in the treatment and control groups must be sacrificed.

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

effects from a chemical, and in the frequency of measures, depending on the duration of potential chemical exposures.

(i) *Hematology.* The recommended parameters are red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, white blood cell count, differential leukocyte count, platelet count, and a measure of clotting potential, such as prothrombin time or activated partial thromboplastin time.

(ii) *Clinical chemistry.* (A) Parameters which are considered appropriate to all studies are electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance and signs of clinical toxicity.

(B) The recommended clinical chemistry determinations are potassium, sodium, glucose, total cholesterol, urea nitrogen, creatinine, total protein and albumin. More than 2 hepatic enzymes, (such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, or gamma glutamyl transpeptidase) should also be measured. Measurements of additional enzymes (of hepatic or other origin) and bile acids, may also be useful.

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

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

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

(10) *Ophthalmological examination.* Ophthalmological examinations using an ophthalmoscope or an equivalent device must be made on all animals prior to the administration of the test substance and on all high dose and con-

trol groups at termination. If changes in the eyes are detected, all animals in the other dose groups must be examined.

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

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

(iii) The following organs and tissues, or representative samples thereof, should be preserved in a suitable medium for possible future histopathological examination:

(A) Digestive system—salivary glands, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, gallbladder (when present).

(B) Nervous system—brain (including sections of medulla/pons, cerebellum and cerebrum), pituitary, peripheral nerve (sciatic or tibial, preferably in close proximity to the muscle), spinal cord (three levels: cervical, mid-thoracic and lumbar), eyes (retina, optic nerve).

(C) Glandular system—adrenals, parathyroid, thyroid.

(D) Respiratory system—trachea, lungs, pharynx, larynx, nose.

(E) Cardiovascular/hemopoietic system—aorta, heart, bone marrow (and/or fresh aspirate), lymph nodes (preferably one lymph node covering the route of administration and another one distant from the route of administration to cover systemic effects), spleen, thymus.

(F) Urogenital system—kidneys, urinary bladder, prostate, testes, epididymides, seminal vesicle(s), uterus, ovaries, female mammary gland.

(G) Others—all gross lesions and masses, skin.

(12) *Histopathology.* (i) The following histopathology must be performed:

(A) Full histopathology on the organs and tissues, listed in paragraph (e)(11)(iii) of this section, of all rodents in the control and high dose groups, and all rodents that died or were sacrificed during the study.

- (B) All gross lesions in all animals.
- (C) Target tissues in all animals.
- (D) When a satellite group is used, histopathology should be performed on tissues and organs identified as showing effects in the treated groups.
  - (ii) If excessive early deaths or other problems occur in the high dose group compromising the significance of the data, the next dose level should be examined for complete histopathology.
  - (iii) An attempt should be made to correlate gross observations with microscopic findings.
  - (iv) Tissues and organs designated for microscopic examination should be fixed in 10% buffered formalin or a recognized suitable fixative as soon as necropsy is performed and no less than 48 hours prior to trimming.
- (f) *Data and reporting*—(1) *Treatment of results.* (i) Data must be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions, the types of lesions and the percentage of animals displaying each type of lesion.
  - (ii) When applicable, all observed results, qualitative and quantitative, should be evaluated by an appropriate and generally accepted statistical method. Any generally accepted statistical methods may be used; the statistical methods, including significance criteria, should be selected during the design of the study.
  - (2) *Evaluation of study results.* The findings of a subchronic oral toxicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the toxic effects and the necropsy and histopathological findings. The evaluation must include the relationship between the dose of the test substance and the presence or absence, the incidence and severity, of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects. A properly conducted subchronic test should provide a satisfactory estimation of a NOEL. It also can indicate the need for an additional longer-term study and provide information on the selection of dose levels.
    - (3) *Test report.* In addition to reporting requirements specified under EPA Good Laboratory Practice Standards at 40 CFR part 792, subpart J, the following specific information must be reported:
      - (i) Test substance characterization should include:
        - (A) Chemical identification.
        - (B) Lot or batch number.
        - (C) Physical properties.
        - (D) Purity/impurities.
      - (ii) Identification and composition of any vehicle used.
      - (iii) Test system should contain data on:
        - (A) Species and strain of animals used and rationale for selection if other than that recommended.
        - (B) Age including body weight data and sex.
        - (C) Test environment including cage conditions, ambient temperature, humidity, and light/dark periods.
        - (D) Identification of animal diet.
        - (E) Acclimation period.
      - (iv) Test procedure should include the following data:
        - (A) Method of randomization used.
        - (B) Full description of experimental design and procedure.
        - (C) Dose regimen including levels, methods, and volume.
      - (v) Test results should include:
        - (A) Group animal data. Tabulation of toxic response data by species, strain, sex and exposure level for:
          - (1) Number of animals exposed.
          - (2) Number of animals showing signs of toxicity.
          - (3) Number of animals dying.
        - (B) Individual animal data. Data should be presented as summary (group mean) as well as for individual animals.
          - (1) Date of death during the study or whether animals survived to termination.
          - (2) Date of observation of each abnormal sign and its subsequent course.
          - (3) Body weight data.
          - (4) Feed and water (if collected) consumption data.
          - (5) Achieved dose (mg/kg/day) as a time-weighted average if the test substance is administered in the diet or drinking water.
          - (6) Results of ophthalmological examination.

(7) Results of hematological tests performed.

(8) Results of clinical chemistry tests performed.

(9) Results of urinalysis, if performed.

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

(11) Detailed description of all histopathological findings.

(12) Statistical treatment of results, where appropriate.

(g) *Quality control.* A system must be developed and maintained to assure and document adequate performance of laboratory equipment. The study must be conducted in compliance with 40 CFR Part 792—Good Laboratory Practice Standards.

(h) *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) Boyd, E.M. Chapter 14. Pilot Studies, 15. Uniposal Clinical Parameters, 16. Uniposal Autopsy Parameters. Predictive Toxicometrics. Williams and Wilkins, Baltimore (1972).

(2) Fitzhugh, O.G. *Subacute Toxicity, Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* The Association of Food and Drug Officials of the United States (1959, 3rd Printing 1975) pp. 26-35.

(3) Organization for Economic Cooperation and Development. OECD guidelines for Testing of Chemicals. Guideline 408: Subchronic Oral Toxicity-Rodent: 90-day Study, Adopted: May 12, 1981.

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

[65 FR 78783, Dec. 15, 2000]

**§ 799.9325 TSCA 90-day dermal toxicity.**

(a) *Scope.* This section is intended to meet the testing requirements under section 4 of the Toxic Substances Con-

trol Act (TSCA). In the assessment and evaluation of the toxic characteristics of a chemical, the determination of subchronic dermal toxicity may be carried out after initial information on toxicity has been obtained by acute testing. The subchronic dermal study has been designed to permit the determination of the no-observed-effects level (NOEL) and toxic effects associated with continuous or repeated exposure to a test substance for a period of 90 days. This study is not capable of determining those effects that have a long latency period for development (e.g., carcinogenicity and life shortening). Extrapolation from the results of this study to humans is valid only to a limited degree. It can, however, provide useful information on the degree of percutaneous absorption, target organs, the possibilities of accumulation, and can be of use in selecting dose levels for chronic studies and for establishing safety criteria for human exposure.

(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 870.3250 (August 1998, final guideline). This source is available at the address in paragraph (h) of this section.

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

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

*Dose* in a subchronic dermal study is the amount of test substance applied daily to the skin for 90 days. Dose is expressed as weight of the test substance (grams, milligrams), per unit body weight of test animal (milligrams per kilogram), or as weight of the test substance per unit of surface area (milligrams per square centimeter) per day.

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