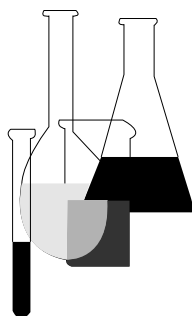




Health Effects Test Guidelines

OPPTS 870.3150 Subchronic Nonrodent Oral Toxicity—90-Day



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading “Environmental Test Methods and Guidelines” or in paper by contacting the OPP Public Docket at (703) 305-5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202-512-1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202-512-0132 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading “Environmental Test Methods and Guidelines.”

OPPTS 870.3150 Subchronic nonrodent oral toxicity—90-day.

(a) **Scope—(1) Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline is OPP 82-1 90-Day Oral—Two Species, Rodent and Nonrodent (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09-82-025, 1982; and OECD 409 Subchronic Oral Toxicity—Nonrodent: 90-Day.

(b) **Purpose.** The determination of subchronic oral toxicity in the assessment and evaluation of the toxic characteristics of a chemical 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-effect level (NOEL) and toxic effects associated with continuous or repeated exposure to a test substance for a period of 90 days. The test is not capable of determining those effects that have a long latency period for development (e.g. carcinogenicity and life shortening). It provides information on health hazards likely to arise from repeated exposure by the oral route over a limited period of time. It provides information on 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.

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

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 is the amount of test substance administered. Dose is expressed as weight of test substance (grams, milligrams) per unit weight of test animal (e.g. milligrams per kilogram), or as weight of test substance per unit weight of food or drinking water.

No-effect-level/No-toxic-effect-level/No-adverse-effect-level/No-observed-effect-level is the maximum dose used in a test which produces no observed adverse effects. A NOEL is expressed in terms of the weight of a substance given daily per unit weight of test animal (milligrams per kilogram). When administered to animals in food or drinking water the NOEL is expressed as milligrams per kilogram of food or milligrams per milliliter of water.

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 percent) of a life span.

(d) **Principle of the test method.** The test substance is administered orally in graduated daily doses to several groups of experimental animals, one dose level per group, for a period of 90 days. During the period of administration the animals are observed daily to detect signs of toxicity. Animals which die during the period of administration are necropsied. At the conclusion of the test all animals are necropsied and histopathological examinations carried out.

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

(f) **Test procedures—(1) Animal selection—(i) Species and strain.** A mammalian species should be used for testing. The commonly used nonrodent species is the dog, preferably of a defined breed; the beagle is frequently used. If other mammalian species are used, the tester should provide justification/reasoning for his or her selection.

(ii) **Age—(A)** Young adult animals should be used. At the commencement of the study the weight variation of animals used should not exceed ± 20 percent of the mean weight for each sex.

(B) In the case of the dog, dosing should commence after acclimation, preferably at 4 to 6 mon and not later than 9 mon of age.

(iii) **Sex.** (A) Equal numbers of animals of each sex should be used at each dose level.

(B) The females should be nulliparous and nonpregnant.

(iv) **Numbers—(A)** At least eight animals (four females and four males) should be used at each dose level.

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

(2) **Control groups.** A concurrent control group is required. This group should 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.

(3) **Dose levels and dose selection.** (i) In subchronic toxicity tests, it is desirable to have a dose response relationship as well as a no-observed-toxic-effect level. Therefore, at least three dose levels with a control and, where appropriate, a vehicle control (corresponding to the concentration of vehicle at the highest exposure level) should 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) There should be no fatalities at the highest dose level.

(iii) The lowest dose level should not produce any evidence of toxicity. The lowest dose level should exceed a usable estimation of human exposure if available.

(iv) The intermediate dose levels should produce minimal observable toxic effects. If more than one intermediate dose is used, the dose levels should be spaced to produce a gradation of toxic effects.

(v) There should be no fatalities in the low and intermediate dose groups.

(4) **Exposure conditions.** The animals are dosed with the test substance on a 7-day per week basis over a period of 90 days. However, based primarily on practical considerations, dosing in gavage or capsule studies on a 5-day per week basis is considered to be acceptable.

(5) **Observation period.** (i) Duration of observation should be for at least 90 days.

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

(6) **Administration of the test substance.** (i) The test substance may be administered in the diet or in capsules.

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

(iii) Where necessary, the test substance is dissolved or suspended in a suitable vehicle. If a vehicle or diluent is needed, it should not elicit important toxic effects itself nor 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, followed by consideration of a solution of oil and by possible solution in other vehicles.

(iv) 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 the BW of the animals should be used; the alternative used should be specified.

(v) For a substance administered by gavage or capsule, the dose should be given at approximately the same time each day, and adjusted at intervals (weekly or biweekly) to maintain a constant dose level in terms of animal BW.

(7) **Observation of animals.** (i) Each animal should be observed daily and, if necessary, handled to appraise its physical condition.

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

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

(iv) Cage-side observations should include, but not be limited to, changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity, and behavior pattern.

(v) Measurements should be made weekly of feed consumption or water consumption when the test substance is administered in the feed or drinking water.

(vi) Animals should be weighed weekly.

(vii) At the end of the 90-day period all survivors in the nonsatellite treatment groups should be sacrificed. Moribund animals should be removed and sacrificed when noticed.

(8) **Clinical examinations.** (i) The following examinations should be made on all animals of each sex in each group.

(A) Certain hematology determinations should be carried out at least two times during the test period on all groups of animals including concurrent controls: After 30 days of test and just prior to terminal sacrifice at the end of the test period. Hematology determinations which are appropriate to all studies: Hematocrit, hemoglobin concentration, erythrocyte count, total and differential leukocyte count, and a measure of clotting potential such as clotting time, prothrombin time, thromboplastin time, or platelet count.

(B) Certain clinical biochemistry determinations on blood should be carried out at least two times during the test period on all groups of animals including concurrent controls—after 30 days of test and just prior

to terminal sacrifice at the end of the test period. Clinical biochemistry test areas 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. Suggested determinations include calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with period of fasting appropriate to the species), serum alanine aminotransferase, as serum aspartate aminotransferase, ornithine decarboxylase, gamma glutamyl transpeptidase, urea nitrogen, albumen, blood creatinine, total bilirubin, and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include analyses of lipids, hormones, acid/base balance, methemoglobin, and cholinesterase activity. Additional clinical biochemistry may be employed, where necessary, to extend the investigation of observed effects.

(ii) The following examinations should be made on high dose and control groups. If changes in the eyes are detected, all animals should be examined.

(A) Ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, should be made prior to the administration of the test substance and at the termination of the study.

(B) Urinalysis is not recommended on a routine basis, and should be carried out only when there is an indication based on expected and or observed toxicity.

(9) **Gross necropsy.** (i) All animals should 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) At least the liver, kidneys, adrenals, and gonads should be weighed wet as soon as possible after dissection to avoid drying. In addition, for the nonrodent, the thyroid with parathyroids also should be weighed wet.

(iii) The following organs and tissues, or representative samples thereof, should be preserved in a suitable medium for possible future histopathological examination: All gross lesions; lungs—which should be removed intact, weighed, and treated with a suitable fixative to ensure that lung structure is maintained (perfusion with the fixative is considered to be an effective procedure); nasopharyngeal tissues; brain—including sections of medulla/pons, cerebellar cortex, and cerebral cortex; pituitary; thyroid/parathyroid; thymus; trachea; heart; sternum with bone marrow; salivary glands; liver; spleen; kidneys; adrenals; pancreas; gonads; uterus; accessory genital organs (epididymis, prostate, and, if present, seminal vesicles); aorta; (skin); gall bladder (if present); esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representa-

tive lymph node; (mammary gland); (thigh musculature); peripheral nerve; (eyes); (femur—including articular surface); (spinal cord at three levels—cervical, midthoracic, and lumbar); and (zygomatic and extraocular lacrimal glands).

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

(i) Full histopathology on the organs and tissues, listed above, of all nonrodents.

(ii) All gross lesions in all animals.

(iii) Target organs in all animals.

(iv) The tissues listed above if indicated by signs of toxicity of target organ involvement.

(v) Lungs, liver, and kidneys of all animals.

(g) **Data and reporting—(1) Treatment of results.** (i) Data should 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) All observed results, quantitative and incidental, should be evaluated by an appropriate statistical method. Any generally accepted statistical methods may be used; the statistical methods should be selected during the design of the study.

(2) **Evaluation of the study results.** (i) 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 will 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, BW changes, effects on mortality and any other general or specific toxic effects. A properly conducted subchronic test should provide a satisfactory estimation of a no-effect-level.

(ii) Further investigation to establish absorption and bioavailability of the test substance should be considered in any study which demonstrates an absence of toxic effects.

(3) **Test report.** In addition to the reporting requirements as specified under 40 CFR part 792, subpart J (Good Laboratory Practice Standards) the following specific information should be reported:

(i) **Group animal data.** Tabulation of toxic response data by species, strain, sex, and exposure level for:

(A) Number of animals dying.

(B) Number of animals showing signs of toxicity.

(C) Number of animals exposed.

(ii) **Individual animal data.** (A) Date of death during the study or whether animals survived to termination.

(B) Date of observation of each abnormal sign and its subsequent course.

(C) BW data.

(D) Feed consumption data when collected.

(E) Hematological tests employed and all results.

(F) Clinical biochemistry tests employed and all results.

(G) Necropsy findings.

(H) Detailed description of all histopathological findings.

(I) Statistical treatment of results where appropriate.

(h) **References.** The following references should be consulted for additional background information on this test guideline.

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

(2) Fitzhugh, O.G., Subacute Toxicity, in *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) Food Safety Council. Subchronic Toxicity Studies, in *Proposed System for Food Safety Assessment*. Food Safety Council, Columbia (1978) pp. 83–96.

(4) National Academy of Sciences. *Principles and Procedures for Evaluating the Toxicity of Household Substances*, a report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(5) World Health Organization. Part I. Environmental Health Criteria 6, in *Principles and Methods for Evaluating the Toxicity of Chemicals*. World Health Organization, Geneva (1978).