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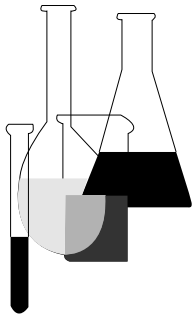
# Health Effects Test Guidelines OPPTS 870.3100 90–Day Oral Toxicity



**“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 870.3100 90-Day oral toxicity.

(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 material used in developing this harmonized OPPTS test guideline are 40 CFR 798.2650 Oral Toxicity; OPP 82–1 90–Day Oral—Two Species, Rodent and Nonrodent; and OECD 408 Subchronic Oral Toxicity—Rodent: 90–Day.

(b) **Purpose.** 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-effect 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 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.

(c) **Definitions.** The definitions in section 3 of the Toxic Substance Control Act (TSCA) and the definitions in 40 CFR Part 792—Good Laboratory Practice Standards 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* in a subchronic oral study is the amount of test substance administered 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 (BW) of test animal (milligram per kilogram) or as weight of the test substance in parts per million in food or drinking water.

*No-observed-effect-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 percent) 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 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 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 mammalian species should be used for testing. A variety of rodent species may be used, although the rat is the preferred species. Commonly used laboratory strains should be employed. Commonly used nonrodent species is the dog. If other mammalian species are used, the tester should provide justification/reasoning for their selection.

(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 weeks of age.

(C) Dosing of dogs should begin between 4 and 6 mon of age and in no case later than 9 mon of age.

(D) At the commencement of the study the weight variation of animals used should not exceed ±20 percent of the mean weight for each sex.

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

(iv) **Numbers.** (A) At least 20 rodents (10 males and 10 females) or 8 nonrodents (4 males and 4 females) 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.

(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 should be assigned a unique identification number. Dead animals, their preserved organs and tissues, and microscopic slides should 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. Dogs should be housed individually.

(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 30 and 70 percent.

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

(E) Control and test animals should 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. Rodents should be fed and watered ad libitum with food replaced at least weekly. For nonrodents feeding should be at least daily and water ad libitum.

(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.

(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, followed by consideration of a solution of 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 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.

(4) **Satellite group.** For rodents, 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) 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) The highest dose level in rodents should result in toxic effects but not produce an incidence of fatalities which would prevent a meaningful evaluation; for nonrodents there should be no fatalities.

(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 is acceptable. If the test substance is administered via capsule (nonrodents), or in the drinking water (rodents), or mixed in the diet (rodents or nonrodents), then exposure should be on a 7–day per week basis.

(ii) All animals should 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 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 body weight.

(7) **Observation period.** The animals should be observed for a period of 90 days. 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 should be made at least once 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).

(ii) A careful clinical examination should 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 motor activity, gait and posture, reactivity to handling or sensory stimuli, grip strength, and stereotypies 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 should be made weekly of food consumption or water consumption if drinking water is the exposure route.

(v) Individual weights of animals should 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 groups should be sacrificed.

(9) **Clinical pathology.** Hematology and clinical chemistry examinations should be made on all animals, including controls, of each sex in each group for rodents and all animals when nonrodents are used as test animals. For rodents, the hematology and clinical chemistry parameters should be examined at terminal sacrifice at the end of the study. For nonrodents, the hematology and clinical chemistry parameters should be examined once prior to initiation of dosing, at monthly intervals or midway through the test period and at the termination.

(i) **Hematology.** The recommended parameters are hemoglobin and hematocrit concentrations, red blood cell count, white blood cell count, differential leukocyte count, platelet count, and a measure of clotting potential such as prothrombin time or 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) Suggested blood clinical chemistry determinations.

(*1*) Electrolytes—calcium, chloride, magnesium, phosphorus, potassium, sodium

(*2*) Enzymes—alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, sorbitol dehydrogenase.

(*3*) Other—albumin, blood creatinine, blood urea nitrogen, globulin, glucose (fasting), total bilirubin, total cholesterol, total serum protein.

(*4*) 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.

(iii) Urinalysis is not recommended on a routine basis, but only when there is an indication based on expected or observed toxicity.

(10) **Optional immunotoxicity screen.** To fulfill, in part, requirements for an immunotoxicity screen, subpopulation of splenic or peripheral blood lymphocytes in the rodents should be enumerated and quantified. Total T-, Total B-, Total T-helper, T-suppressor/cytotoxic and Natural Killer (NK) cell populations should be determined on at least 10 rodents of each sex in each group at the end of 90 days.

(11) **Ophthalmological examination.** Ophthalmological examinations using an ophthalmoscope or an equivalent device should be made on all animals prior to the administration of the test substance and on all high dose and control groups at termination. If changes in the eyes are detected, all animals in the other dose groups should be examined.

(12) **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) In both the rodents and nonrodents the liver, lungs, brain, kidneys, spleen, and gonads should be trimmed and weighed wet, as soon as possible after dissection. In addition, the thyroid with parathyroids in nonrodents should be weighed.

(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, gall bladder (dogs).

(B) Nervous system—brain (multiple sections), pituitary, peripheral nerve(s), spinal cord (three levels), eyes (retina, optic nerve).

(C) Glandular system—adrenals, parathyroids, thyroids.

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

(E) Cardiovascular/hemopoietic system—aorta (thoracic), heart, bone marrow, lymph nodes, spleen, thymus.

(F) Urinogenital system—kidneys, urinary bladder, prostate, testes, epididymides, seminal vesicle(s), uterus, ovaries.

(G) Others—all gross lesions and masses, sternum and/or femur.

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

(A) Full histopathology on the organs and tissues, listed under paragraph (e)(12)(iii) of this guideline, of all rodents in the control and high dose groups, all nonrodents, and all rodents that died or were killed during the study.

(B) All gross lesions in all animals.

(C) Target tissues in all animals.

(D) Lungs, liver, and kidneys of all animals. Special attention to examination of the lungs of rodents should be made for evidence of infection as this provides a convenient assessment of the state of health of the animals.

(E) When a satellite group is used (rodents), 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 percent buffered formalin or a recognized suitable fixative as soon as necropsy is performed and no less than 48 h prior to trimming. Tissues should be trimmed to a maximum thickness of 0.4 cm for processing.

(f) **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 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 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, 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 and 40 CFR part 160, and the OECD principles of GLP (ISBN 92–64–12367–9), the following specific information should be reported:

(i) Test substance characterization should include:

(A) Chemical identification.

(B) Lot or batch number.

(C) Physical properties.

(D) Purity/impurities.

(E) Identification and composition of any vehicle used.

(ii) 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.

(iii) 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.

(iv) 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 consumption data, when collected.

(*5*) Results of ophthalmological examination, when performed.

(*6*) Results of hematological tests performed.

(*7*) Results of clinical chemistry tests performed.

(*8*) Results of immunotoxicity screen, when performed.

(*9*) Necropsy findings, including absolute and relative organ weight data.

(*10*) Detailed description of all histopathological findings.

(*11*) Statistical treatment of results, where appropriate.

(g) **Quality control.** A system should be developed and maintained to assure and document adequate performance of laboratory staff and equipment. The study must be conducted in compliance with GLP regulations..

(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. 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. 260935.

(3) Food Safety Council. Subchronic Toxicity Studies, Proposed System for Food Safety Assessment. (Columbia: Food Safety Council, 1978) pp. 830996.

(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) 0rganization for Economic Co-operation and Development. Guidelines for Testing of Chemicals, Section 4-Health Effects, Part 408 Subchronic Toxicity Studies, Paris, 1981.

(6) United States Environmental Protection Agency. Office of Testing and Evaluation. Proposed Health Effects Test Standards for Toxic Substances Control Act Test Rules. 40 CFR Part 772. Standard for Development of Test Data. Subpart D. FEDERAL REGISTER Vol. 44, pp. 27350-27362.

(7) World Health Organization. Guidelines for Evaluation of Drugs for Use in Man, WHO Technical Report Series No. 563. (Geneva: World Health Organization, 1975).

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

(9) World Health Organization. Principles for Pre-Clinical Testing of Drug Safety, WHO Technical Report Series No. 341. (Geneva: World Health Organization, 1966).