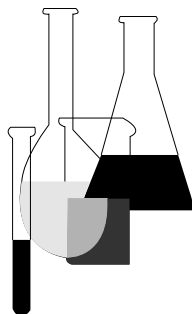




Health Effects Test Guidelines

OPPTS 870.4100 Chronic Toxicity



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.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512-0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (<http://www.epa.gov/epahome/research.htm>) under the heading "Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines."

OPPTS 870.4100 Chronic 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 798.3260 Chronic Toxicity; OPP 83-1 Chronic Feeding—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 452 Chronic Toxicity studies.

(b) **Purpose.** The objective of a chronic toxicity study is to determine the effects of a substance in a mammalian species following prolonged and repeated exposure. A chronic toxicity study should generate data from which to identify the majority of chronic effects and to define long-term dose-response relationships. The design and conduct of chronic toxicity tests should allow for the detection of general toxic effects, including neurological, physiological, biochemical, and hematological effects and exposure-related morphological (pathological) effects.

(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.

Chronic toxicity is the adverse effects occurring as a result of the repeated daily exposure of experimental animals to a chemical by the oral, dermal, or inhalation routes of exposure.

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 chronic toxicity study is the amount of test substance administered daily via the oral, dermal or inhalation routes for a period of at least 12 months. 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 (ppm) in food or drinking water per day. For inhalation exposure, dose is expressed as weight of the test substance per unit volume of air (milligrams per liter) or as parts per million per day. For dermal exposure, dose is expressed as weight of the test substance (grams, milligrams) per unit body weight of the test animal (milligrams per kilogram) or as weight of the substance per unit of surface area (milligrams per square centimeter) per day.

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).

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 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.

(e) **Test procedures—(1) Animal selection—(i) Species and strain.** Testing should be performed with two mammalian species, one a rodent and the other a nonrodent. The rat is the preferred rodent species and the dog is the preferred nonrodent species. Commonly used laboratory strains should be employed. 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 months of age and in no case later than 9 months of age.

(D) At commencement of the study, the weight variation of animals used should be within 20 percent of the mean weight for each sex.

(E) Studies using prenatal or neonatal animals may be recommended under special conditions.

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

(B) Females should be nulliparous and nonpregnant.

(iv) **Numbers.** (A) For rodents, at least 40 animals (20 males and 20 females) and for nonrodents (dogs) at least 8 animals (4 females and 4 males) should be used at each dose level and concurrent control group.

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

(C) The number of animals at the termination of the study must be adequate for a meaningful and valid statistical evaluation of chronic ef-

fects. The Agency must be notified if excessive early deaths or other problems are encountered that might compromise the integrity of the study.

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

(E) 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 unique numbers assigned.

(v) **Husbandry.** (A) Rodents 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. Rodents should be housed individually in dermal studies and during exposure in inhalation studies. Caging should be appropriate to the nonrodent species. However, it is recommended that dogs are housed individually.

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

(C) The relative humidity of the experimental animal rooms should be 50 ± 20 percent.

(D) Where lighting is artificial, the sequence should be 12 hours light/12 hours 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. Animals should be fed and watered ad libitum with food replaced at least weekly.

(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 5 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 it should not elicit toxic effects itself nor substantially alter the chemical or toxicological properties of the test substance. It is recommended that wherever possible the use of an aqueous solution be the first choice, followed by consideration of solution in oil, and finally, solution in other vehicles.

(ii) One lot of the test substance should be used, if possible, 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.** A satellite group of 40 animals (20 animals per sex) for rodents and 8 animals (4 animals per sex) for nonrodents may be treated with the high-dose level for 12 months and observed for reversibility, persistence, or delayed occurrence of toxic effects for a post-treatment of appropriate length, normally not less than 28 days. In addition, a control group of 40 animals (20 animals per sex) for rodents and 8 animals (4 animals per sex) for nonrodents should be added to the satellite study.

(5) **Dose levels and dose selections.** (i) In chronic toxicity tests, it is desirable to determine a dose-response relationship as well as a NOEL. Therefore, at least three dose levels with a control group and, where appropriate, a vehicle control (corresponding to the concentration of the vehicle at the highest exposure level) should be used. Dose levels should be spaced to produce a gradation of effects. A rationale must be provided for the doses selected.

(ii) The highest-dose level should elicit signs of toxicity without substantially altering the normal life span of the animal. The highest dose should be determined based on the findings from a 90-day study to ensure that the dose used is adequate to assess the chronic toxicity of the test substance. Thus, the selection of the highest dose to be tested is dependent upon changes observed in several toxicological parameters in subchronic studies. The highest dose tested need not exceed 1,000 mg/kg/day. If dermal application of the test substance produces severe skin irritation, then it may be necessary either to terminate the study and choose a lower high-dose level or to reduce the dose level. Gross criteria for defining severe irritation would include ulcers, fissures, exudate/crust(eschar), dead tissue,

or anything leading to destruction of the functional integrity of the epidermis (e.g. caking, open sores, fissuring, eschar). Histological criteria for defining severe irritation would include follicular and interfollicular crust, microulcer, mild/moderate degeneration/necrosis, moderate/marked epidermal edema, marked dermal edema, and marked inflammation.

(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.** The three main routes of administration are oral, dermal, and inhalation. The choice of the route of administration depends upon the physical and chemical characteristics of the test substance and the form typifying exposure in humans.

(i) **Oral studies.** Ideally, the animals should be dosed by gavage or with capsules on a 7-day per week basis for a period of at least 12 months. However, based primarily on practical considerations, dosing by gavage or capsules on a 5-day per week schedule is acceptable. If the test substance is administered via in the drinking water or mixed in the diet, exposure should be on a 7-day per week basis.

(ii) **Dermal studies.** (A) Preparation of animal skin. Shortly before testing, fur should be clipped from not less than 10 percent of the body surface area for application of the test substance. In order to dose approximately 10 percent of the body surface, the area starting at the scapulae (shoulders) to the wing of the ileum (hipbone) and half way down the flank on each side of the animal should be shaved. Shaving should be carried out approximately 24 hours before dosing. Repeated clipping or shaving is usually needed at approximately weekly intervals. When clipping or shaving the fur, care should be taken to avoid abrading the skin which could alter its permeability.

(B) Preparation of test substance. Liquid test substances are generally used undiluted, except as indicated in paragraph (e)(5)(ii) of this guideline. Solids should be pulverized when possible. The substance should be moistened sufficiently with water or, when necessary, with a suitable vehicle to ensure good contact with the skin. When a vehicle is used, the influence of the vehicle on toxicity of, and penetration of the skin by, the test substance should be taken into account. The volume of application should be kept constant, e.g. less than 100 μL for the mouse and less than 300 μL for the rat. Different concentrations of test solution should be prepared for different dose levels.

(C) Administration of test substance. The duration of exposure should be at least for 12 months. Ideally, the animals should be treated with test substance for at least 6 h/day on a 7-day per week basis. However, based on practical considerations, application on a 5-day per week basis is ac-

ceptable. Dosing should be conducted at approximately the same time each day. The test substance should be applied uniformly over the treatment site. The surface area covered may be less for highly toxic substances. As much of the area should be covered with as thin and uniform a film as possible. For rats, the test substance may be held in contact with the skin with a porous gauze dressing and nonirritating tape if necessary. The test site should be further covered in a suitable manner to retain the gauze dressing plus test substance and to ensure that the animals cannot ingest the test substance. The application site should not be covered when the mouse is the species of choice. The test substance may be wiped from the skin after the six-hour exposure period to prevent ingestion.

(iii) *Inhalation studies.* (A) The animals should be exposed to the test substance for 6 h/day on a 7-day per week basis, for a period of at least 12 months. However, based primarily on practical considerations, exposure for 6 hours per day on a 5-day per week basis is acceptable.

(B) The animals should be tested in dynamic inhalation equipment designed to sustain a minimum air flow of 10 air changes per hour, an adequate oxygen content of at least 19 percent, and uniform conditions throughout the exposure chamber. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into surrounding areas. It is not normally necessary to measure chamber oxygen concentration if airflow is adequate.

(C) The selection of a dynamic inhalation chamber should be appropriate for the test substance and test system. When a whole body chamber is used, individual housing must be used to minimize crowding of the test animals and maximize their exposure to the test substance. To ensure stability of a chamber atmosphere, the total volume occupied by the test animals should not exceed 5 percent of the volume of the test chamber. It is recommended, but not required, that nose-only or head-only exposure be used for aerosol studies in order to minimize oral exposures due to animals licking compound off their fur. The animals should be acclimated and heat stress minimized.

(D) The temperature at which the test is performed should be maintained at 22 ± 2 °C. The relative humidity should be maintained between 40–60 percent, but in certain instances (e.g., use of water vehicle) this may not be practicable.

(E) The rate of air flow should be monitored continuously but recorded at least three times during the exposure.

(F) Temperature and humidity should be monitored continuously but should be recorded at least every 30 min.

(G) The actual concentrations of the test substance should be measured in the breathing zone. During the exposure period, the actual con-

centrations of the test substance should be held as constant as practicable, monitored continuously or intermittently depending on the method of analysis. Chamber concentration may be measured using gravimetric or analytical methods, as appropriate. If trial run measurements are reasonably consistent (± 10 percent for liquid aerosol, gas, or vapor; ± 20 percent for dry aerosol), then two measurements should be sufficient. If measurements are not consistent, three to four measurements should be taken. Whenever the test substance is a formulation, or it is necessary to formulate the test substance with a vehicle for aerosol generation, the analytical concentration must be reported for the total formulation, and not just for the active ingredient (AI). If, for example, a formulation contains 10 percent AI and 90 percent inerts, a chamber of analytical limit concentration of 2 mg/L would consist of 0.2 mg/L of the AI. It is not necessary to analyze inert ingredients provided the mixture at the animal's breathing zone is analogous to the formulation; the grounds for this conclusion must be provided in the study report. If there is some difficulty measuring chamber analytical concentration due to precipitation, nonhomogeneous mixtures, volatile components, or other factors, additional analysis of inert components may be necessary.

(H) During the development of the generating system, particle size analysis should be performed to establish the stability of aerosol concentrations with respect to particle size. The mass median aerodynamic diameter (MMAD) particle size range should be between 1–3 μm . The particle size of hygroscopic materials should be small enough when dry to assure that the size of the swollen particle will still be within the 1–3 μm range. Measurements of aerodynamic particle size in the animal's breathing zone should be measured during a trial run. If MMAD values for each exposure level are within 10 percent of each other, then two measurements during the exposures should be sufficient. If pretest measurements are not within 10 percent of each other, three to four measurements should be taken.

(I) Feed should be withheld during exposure. Water may also be withheld during exposure.

(7) **Observation period.** (i) Animals should be observed for a period of at least 12 months.

(ii) Animals in a 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 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 consid-

eration the peak period of anticipated effects after dosing. The clinical condition of the animal should be recorded.

(ii) A careful clinical examination should be made at least once prior to the initiation of treatment (to allow for within subject comparisons) and once weekly during treatment in all animals. These observations should be made outside the home cage, preferably in a standard arena, and at similar times on each occasion. Effort should be made to ensure that variations in the observation conditions are minimal. Observations should be detailed and carefully recorded, preferably using scoring systems, explicitly defined by the testing laboratory. Signs noted should include, but not be limited to, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backwards) should be recorded.

(iii) Once, near the end of the first year of the exposure period and in any case not earlier than in month 11, assessment of motor activity, grip strength, and sensory reactivity to stimuli of different types (e.g., visual, auditory, and proprioceptive stimuli) should be conducted in rodents. Further details of the procedures that could be followed are described in the references listed under paragraphs (h)(2), (h)(6), (h)(8), (h)(9), (h)(10), and (h)(17) of this guideline.

(iv) Functional observations conducted towards the end of the study may be omitted when data on functional observations are available from other studies and the daily clinical observations did not reveal any functional deficits.

(v) Exceptionally, functional observations may be omitted for groups that otherwise reveal signs of toxicity to an extent that would significantly interfere with functional test performance.

(vi) Body weights should be recorded individually for all animals once prior to the administration of the test substance, once a week during the first 13 weeks of study and at least once every 4 weeks thereafter, unless signs of clinical toxicity suggest more frequent weighing to facilitate monitoring of health status.

(vii) Measurements of feed consumption should be determined weekly during the first 13 weeks of the study and at approximately monthly intervals thereafter unless health status or body weight changes dictate otherwise. Measurements of water consumption should be determined at the same intervals if the test substance is administered in the drinking water.

(viii) Moribund animals should be removed and sacrificed when noticed and the time of death should be recorded as precisely as possible. All survivors should be sacrificed at the end of the study period.

(9) **Clinical pathology.** Hematology, clinical chemistry, and urinalysis should be performed on 10 rats per sex per group, and on all nonrodents. In rodents, the parameters should be examined at approximately 6 month intervals during the conduct of the study and at termination. If possible, these collections should be from the same animals at each interval. In nonrodents, the parameters should be examined once or twice prior to initiation of treatment, at 6-month intervals during the conduct of the study, and at termination. If hematological and biochemical effects were seen in the subchronic study, testing should also be performed at 3 months. Overnight fasting of animals prior to blood sampling is recommended.

(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, calcium (nonrodent), phosphorus (nonrodent), chloride (nonrodent), glucose, total cholesterol, urea nitrogen, creatinine, total protein, total bilirubin (nonrodent), and albumin. More than two 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) **Urinalysis.** Urinalysis for rodents should be performed at the end of the study using timed urine collection. Urinalysis for nonrodents should be performed prior to treatment, midway through treatment and at the end of the study using timed urine collection. Urinalysis determina-

tions include: appearance, volume, osmolality or specific gravity, pH, protein, glucose, and blood/blood cells.

(10) **Ophthalmological examination.** Examinations should be made of all animals using an ophthalmoscope or equivalent device prior to the administration of the test substance and at termination of the study on 10 rats of each sex in the high-dose and control groups and preferably in all nonrodents, but at least the control and high-dose groups should be examined. If changes in eyes are detected, all animals should be examined.

(11) **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, testes, epididymides, ovaries, uterus, nonrodent thyroid (with parathyroid), spleen, brain, and heart should be weighed wet as soon as possible after dissection to avoid drying. The lungs should be weighed if the test substance is administered by the inhalation route.

(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 (multiple sections, including cerebrum, cerebellum and medulla/pons), 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/hematopoietic 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.

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

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

(iv) In inhalation studies, the entire respiratory tract, including nose, pharynx, larynx, and paranasal sinuses should be examined and preserved.

In dermal studies, skin from treated and adjacent control skin sites should be examined and preserved.

(v) Inflation of lungs and urinary bladder with a fixative is the optimal method for preservation of these tissues. The proper inflation and fixation of the lungs in inhalation studies is considered essential for appropriate and valid histopathological examination.

(vi) Information from clinical pathology and other in-life data should be considered before microscopic examination, since they may provide significant guidance to the pathologist.

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

(A) Full histopathology on the organs and tissues (listed under paragraph (e)(11)(iii) of this guideline) of all rodents and nonrodents in the control and high-dose groups, and all rodents and nonrodents that died or were killed during the study. The examination should be extended to all animals in all dosage groups if treatment-related changes are observed in the high-dose group.

(B) All gross lesions in all animals.

(C) Target tissues in all animals.

(ii) If the results show substantial alteration of the animal's normal life span, or other effects that might compromise the significance of the data, the next lower levels should be examined fully as described in paragraph (e)(12)(i) of this guideline.

(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 hours prior to trimming.

(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) When applicable, all observed results (quantitative and qualitative) 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 chronic toxicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the toxic effects as well as the necropsy and histopathological findings. The evaluation will include the relationship between the dose of the test substance and the presence, 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.

(3) **Test report.** In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, 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.

(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.

(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) Time of death during the study or whether animals survived to termination.

(2) Time 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 examinations.

(7) Results of hematological tests performed.

(8) Results of clinical chemistry tests performed.

(9) Urinalysis tests performed and results.

(10) Results of observations made.

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

(12) Detailed description of all histopathological findings.

(13) Statistical treatment of results, where appropriate.

(iv) In addition, for inhalation studies the following should be reported:

(A) Test conditions. The following exposure conditions must be reported:

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

(2) The equipment for measuring temperature, humidity, and particulate aerosol concentrations and size should be described.

(B) Exposure data. These data should be tabulated and presented with mean values and a measure of variability (e.g., standard deviation) and should include:

(1) Airflow rates through the inhalation equipment.

(2) Temperature and humidity of air.

(3) Actual (analytical or gravimetric) concentration in the breathing zone.

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

(5) Particle size distribution, calculated MMAD, and geometric standard deviation (GSD).

(6) Explanation as to why the desired chamber concentration and/or particle size could not be achieved (if applicable) and the efforts taken to comply with this aspect of the guidelines.

(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 as described by the Agency (40 CFR parts 160 and 792), and the OECD principles of GLP (ISBN 92-64-12367-9).

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

(1) Benitz, K.F. Measurement of Chronic Toxicity. *Methods of Toxicology*. Ed. G.E. Paget. Blackwell, Oxford. pp. 82-131 (1970).

(2) Crofton K.M., Howard J.L., Moser V.C., Gill M.W., Leiter L.W., Tilson H.A., MacPhail, R.C. Interlaboratory Comparison of Motor Activity Experiments: Implication for Neurotoxicological Assesments. *Neurotoxicol. Teratol.* 13, 599-609. (1991)

(3) D'Aguanno, W. Drug Safety Evaluation--Pre-Clinical Considerations. *Industrial Pharmacology: Neuroleptic*. Vol. I, Ed. S. Fielding and H. Lal. Futura, Mt. Kisco, NY. pp. 317-332 (1974).

(4) Fitzhugh, O.G. Chronic Oral Toxicity, Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Association of Food and Drug Officials of the United States. pp. 36-45 (1959, 3rd Printing 1975).

(5) Food Safety Council. Proposed System for Food Safety Assessment. Prepared by the Scientific Committee, Food Safety Council. *Food and Cosmetic Toxicology*. Vol. 16, Supplement 2. (December 1978).

- (6) Gad S.C. A Neuromuscular Screen for Use in Industrial Toxicology. *Journal of Toxicology and Environmental Health*. 9, 691–704. (1982)
- (7) Goldenthal, E.I. and D’Aguanno, W. Evaluation of Drugs, Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. The Association of Food and Drug Officials of the United States. pp. 60–67 (1959, 3rd Printing 1975).
- (8) International Programme on Chemical Safety. Principles and Methods for the Assessment of Neurotoxicity Associated with Exposure to Chemicals. Environmental Health Criteria Document No. 60. (1986)
- (9) Meyer O.A., Tilson H.A., Byrd W.C., Riley M.T. A Method for the Routine Assessment of Fore- and Hind-Limb Grip Strength of Rats and Mice. *Neurobehav. Toxicol.* 1, 233–236. (1979)
- (10) Moser V.C., McDaniel K.M., Phillips P.M. Rat Strain and Stock Comparisons using a Functional Observational Battery: Baseline Values and Effects of Amitraz. *Toxicol. Appl. Pharmacol.* 108, 267–283 (1991)
- (11) 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).
- (12) National Center for Toxicological Research. Appendix B, Report of Chronic Studies Task Force Committee, April 13–21, 1972. National Center for Toxicological Research, Rockville, MD (1972).
- (13) Organization for Economic Cooperation and Development. Guidelines for Testing of Chemicals, Section 4-Health Effects, Part 452 Chronic Toxicity Studies, Paris (1981).
- (14) Page, N.P. Chronic Toxicity and Carcinogenicity Guidelines. *Journal of Environmental Pathology and Toxicology*. 11:161–182 (1977).
- (15) Schwartz, E. Toxicology of Neuroleptic Agents. *Industrial Pharmacology: Neuroleptic*. S. Fielding and H. Lal. Futura, Mt. Kisco, NY pp. 203–221 (1974).
- (16) Toxicity and Clinical Trial Subcommittee, Committee on Safety of Medicine. (November 1977).
- (17) Tupper, D.E., Wallace R.B. Utility of the Neurologic Examination in Rats. *Acta. Neurobiol. Exp.* 40, 999–1003 (1980).
- (18) United States Pharmaceutical Manufacturers Association. Guidelines for the Assessment of Drug and Medical Device Safety in Animals. (1977).

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

(20) World Health Organization (WHO). Guidelines for Evaluation of Drugs for Use in Man. WHO Technical Report Series No. 563.

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

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