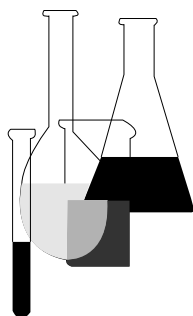




Health Effects Test Guidelines OPPTS 870.3600 Inhalation Developmental Toxicity Study



“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 870.3600 Inhalation developmental toxicity study.

(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 is 40 CFR 798.4350 Inhalation Developmental Toxicity Study.

(b) **Purpose.** In the assessment and evaluation of the toxic characteristics of an inhalable material such as a gas, volatile substance, or aerosol/particulate, determination of the potential developmental toxicity is important. The inhalation developmental toxicity study is designed to provide information on the potential hazard to the unborn which may arise from exposure of the mother during pregnancy.

(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.

Aerodynamic diameter applies to the behavioral size of particles of aerosols. It is the diameter of a sphere of unit density which behaves aerodynamically like the particles of the test substance. It is used to compare particles of different sizes, shapes, and densities and to predict where in the respiratory tract such particles may be deposited. This term is used in contrast to “optical,” “measured” or “geometric” diameters which are representation of actual diameters which in themselves cannot be related to deposition within the respiratory tract.

Concentration refers to an exposure level. Exposure is expressed as weight or volume of test substance per volume of air (milligrams per liter), or as parts per million (ppm).

Developmental toxicity is the property of a chemical that causes in utero death, structural or functional abnormalities or growth retardation during the period of development.

Geometric mean diameter or *median diameter* is the calculated aerodynamic diameter which divides the particles of an aerosol in half based on the weight of the particles. Fifty percent of the particles by weight will be larger than the median diameter and 50 percent of the particles will be smaller than the median diameter. The median diameter and its geometric standard deviation are used to statistically describe the particle size distribution of any aerosol based on the weight and size of the particles.

Inhalable diameter refers to that aerodynamic diameter of a particle which is considered to be inhalable for the organism. It is used to refer

to particles which are capable of being inhaled and may be deposited anywhere within the respiratory tract from the trachea to the deep lung (the alveoli). For man, the inhalable diameter is considered here as 15 μm or less.

No-observed-effect level (NOEL) is the maximum concentration in a test which produces no observed adverse effects. A NOEL is expressed in terms of weight or volume of test substance given daily per unit volume of air.

(d) **Principle of the test method.** The test substance is administered in graduated concentrations, for at least that part of the pregnancy covering the major period of organogenesis, to several groups of pregnant experimental animals, one exposure level being used per group. Shortly before the expected date of delivery, the pregnant females are sacrificed, the uteri removed, and the contents examined for embryonic or fetal deaths, and live fetuses.

(e) **Limit test.** If a test at an exposure of 5 mg/1 (actual concentration of respirable substances) or, where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration, produces no observable developmental toxicity, then a full study using three exposure levels might not be necessary.

(f) **Test procedures**—(1) **Animal selection**—(i) **Species and strain.** Testing should be performed in at least two mammalian species. Commonly used species include the rat, mouse, rabbit, and hamster. If other mammalian species are used, the tester should provide justification/reasoning for their selection. Commonly used laboratory strains should be employed. The strain should not have low fecundity and should preferably be characterized for its sensitivity to developmental toxins.

(ii) **Age.** Young adult animals (nulliparous females) should be used.

(iii) **Sex.** Pregnant female animals should be used at each exposure level.

(iv) **Number of animals.** At least 20 pregnant rats, mice, or hamsters or 12 pregnant rabbits are required at each exposure level. The objective is to ensure that sufficient pups are produced to permit meaningful evaluation of the potential developmental toxicity of the test substance.

(2) **Control group.** A concurrent control group should be used. This group should be exposed to clean, filtered air under conditions identical to those used for the group exposed to the substance of interest. In addition, a vehicle-exposed group may be necessary when the substance under study requires a vehicle for delivery. It is recommended that during preliminary range finding studies, air vs. vehicle exposure be compared. If there is no substantial difference, air exposure itself would be an appro-

appropriate control. If vehicle and air exposure yield different results, both vehicle and air exposed control groups are recommended.

(3) **Concentration levels and concentration selection.** (i) At least three concentration levels with a control and, where appropriate, a vehicle control, should be used.

(ii) The vehicle should neither be developmentally toxic nor have effects on reproduction.

(iii) To select the appropriate concentration levels, a pilot or trial study may be advisable. Since pregnant animals have an increased minute ventilation as compared to non-pregnant animals, it is recommended that the trial study be conducted in pregnant animals. Similarly, since presumably the minute ventilation will vary with progression of pregnancy, the animals should be exposed during the same period of gestation as in the main study. In the trial study, the concentration producing embryonic or fetal lethality or maternal toxicity should be determined.

(iv) Unless limited by the physical/chemical nature or biological properties of the substance, the highest concentration level should induce some overt maternal toxicity such as reduced body weight or body weight gain, but not more than 10 percent maternal deaths.

(v) The lowest concentration level should not produce any grossly observable evidence of either maternal or developmental toxicity.

(vi) Ideally, the intermediate concentration level(s) should produce minimal observable toxic effects. If more than one intermediate concentration is used, the concentration levels should be spaced to produce a gradation of toxic effects.

(4) **Exposure duration.** The duration of exposure should be at least six hours daily allowing appropriate additional time for chamber equilibrium.

(5) **Observation period.** Day 0 in the test is the day on which a vaginal plug and/or sperm are observed. The exposure period should cover the period of major organogenesis. This may be taken as days 6 to 15 for rat and mouse, 6 to 14 for hamster, or 6 to 18 for rabbit.

(6) **Inhalation exposure.** (i)(A) The animals should be tested in inhalation equipment designed to sustain a minimum dynamic air flow of 12 to 15 air changes per hour and ensure an adequate oxygen content of 19 percent and an evenly distributed exposure atmosphere. Where a chamber is used, its design should minimize crowding of the test animals and maximize their exposure to the test substance. This is best accomplished by individual caging. To ensure stability of a chamber atmosphere, the total "volume" of the test animals should not exceed 5 percent of the volume of the test chamber.

(B) Pregnant animals should not be subjected to beyond the minimum amount of stress. Since whole-body exposure appears to be the least stressful mode of exposure, it is the method preferred. In general oro-nasal or head-only exposure, which is sometimes used to avoid concurrent exposure by the dermal or oral routes, is not recommended because of the associated stress accompanying the restraining of the animals. However, there may be specific instances where it may be more appropriate than whole-body exposure. The tester should provide justification/reasoning for its selection.

(ii) A dynamic inhalation system with a suitable flow control system should be used. The rate of air flow should be adjusted to ensure that conditions throughout the exposure chamber are essentially the same. Test material distribution should be established before animals are committed to dosing. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas.

(iii) The temperature at which the test is performed should be maintained at 22 ± 2 °C for rodents or 20 ± 3 °C for rabbits. Ideally, the relative humidity should be maintained between 40 to 60 percent, but in certain instances (e.g. tests of aerosols, use of water vehicle) this may not be practicable.

(7) **Physical measurements.** Measurements or monitoring should be made of the following:

(i) The rate of airflow should be monitored continuously but should be recorded at least every 30 minutes.

(ii) The actual concentration of the test substance should be measured in the breathing zone. During the exposure period the actual concentrations of the test substance should be held as constant as practicable, monitored continuously or intermittently depending on the method of analysis and measured at least at the beginning, at an intermediate time and at the end of the exposure period.

(iii) 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. During exposure, analysis should be conducted as often as necessary to determine the consistency of particle size distribution.

(iv) Temperature and humidity should be monitored continuously and be recorded at least every 30 minutes.

(8) **Food and water during exposure period.** Food should be withheld during exposure. Water may or may not be withheld. If it is not withheld it should not come in direct contact with the test atmospheres.

(9) **Observation of animals.** (i) A gross examination should be made at least once each day.

(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 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, the degree and duration.

(iv) Cage-side observations should include, but not be limited to: Changes in skin and fur, eye and mucous membranes, as well as respiratory, autonomic and central nervous systems, somatomotor activity and behavioral pattern. Particular attention should be directed to observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma.

(v) Measurements should be made weekly of food consumption for all animals in the study.

(vi) Animals should be weighed at least weekly.

(vii) Females showing signs of abortion or premature delivery should be sacrificed and subjected to a thorough macroscopic examination.

(10) **Gross necropsy.** (i) At the time of sacrifice or death during the study, the dam should be examined macroscopically for any structural abnormalities or pathological changes which may have influenced the pregnancy.

(ii) Immediately after sacrifice or death, the uterus should be removed, weighed, and the contents examined for embryonic or fetal deaths and the number of viable fetuses. Gravid uterine weights should not be obtained from dead animals if autolysis or where decomposition has occurred. The degree of resorption should be described in order to help estimate the relative time of death.

(iii) The number of corpora lutea should be determined for all species except mice.

(iv) The sex of the fetuses should be determined and they should be weighed individually, the weights recorded, and the mean fetal weight derived.

(v) Following removal, each fetus should be examined externally.

(vi) For rats, mice and hamsters, one-third to one-half of each litter should be prepared and examined for skeletal anomalies, and the remaining part of each litter should be prepared and examined for soft tissue anomalies using appropriate methods.

(vii) For rabbits, each fetus should be examined by careful dissection for visceral anomalies and then examined for skeletal anomalies.

(g) **Data and reporting**—(1) **Treatment of results.** 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 pregnant animals, the number and percentages of live fetuses and the number of fetuses with any soft tissue or skeletal abnormalities.

(2) **Evaluation of results.** The findings of a developmental toxicity study should be evaluated in terms of the observed effects and the exposure levels producing effects. It is necessary to consider the historical developmental toxicity data on the species/strain tested. A properly conducted developmental toxicity study should provide a satisfactory estimation of a no-effect level.

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

(i) **Test conditions.** (A) Description of exposure apparatus including design, type, dimensions, source of air, system for generating particulates and aerosols, methods of conditioning air, and the method of housing the animals in a test chamber when this apparatus is used.

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

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

(A) Airflow rates through the inhalation equipment.

(B) Temperature of air.

(C) Nominal concentration—total amount of test substance fed into the inhalation equipment divided by volume of air (no standard deviation).

(D) Measured total concentrations (particulate and/or gaseous phases) in test breathing zone.

(E) Particle size distribution (e.g. median aerodynamic diameter of particles with geometric standard deviation) including estimates of the percents of inhalable and non-inhalable portions for the test animals.

(iii) **Animal data.** (A) Toxic response data by concentration.

(B) Species and strain.

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

(D) Date of onset and duration of each abnormal sign and its subsequent course.

(E) Feed, body weight and uterine weight data.

(F) Pregnancy and litter data.

(G) Fetal data (live/dead, sex, soft tissue and skeletal defects, resorptions).

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

(1) Department of Health and Welfare. *The Testing of Chemicals for Carcinogenicity, Mutagenicity and Teratogenicity*. Minister of Health and Welfare. Department of Health and Welfare, Canada (1975).

(2) 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).

(3) World Health Organization. *Principles for the Testing of Drugs for Teratogenicity*. WHO Technical Report Series No. 364. World Health Organization, Geneva (1967).