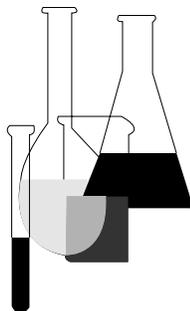




Health Effects Test Guidelines OPPTS 870.3700 Prenatal Developmental Toxicity Study



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 ASCII and 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 870.3700 Prenatal 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.*), as amended by the Food Quality Protection Act (FQPA)(Pub. L.104–170), 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 the OPPT guideline under 40 CFR 798.4900, OPP guideline 83–3, and OECD guideline 414.

(b) **Purpose.** This guideline for developmental toxicity testing is designed to provide general information concerning the effects of exposure of the pregnant test animal on the developing organism; this may include death, structural abnormalities, or altered growth and an assessment of maternal effects. For information on testing for functional deficiencies and other postnatal effects, the guidelines for the two-generation reproductive toxicity study and the developmental neurotoxicity study should be consulted.

(c) **Good laboratory practice standards.** The study should be conducted in accordance with the laboratory practices stipulated in 40 CFR Part 160 (FIFRA) and 40 CFR Part 792 (TSCA)—Good Laboratory Practice Standards.

(d) **Principle of the test method.** The test substance is administered to pregnant animals at least from implantation to one day prior to the expected day of parturition. Shortly before the expected date of delivery, the pregnant females are terminated, the uterine contents are examined, and the fetuses are processed for visceral and skeletal evaluation.

(e) **Test procedures**—(1) **Animal selection**—(i) **Species and strain.** It is recommended that testing be performed in the most relevant species, and that laboratory species and strains which are commonly used in prenatal developmental toxicity testing be employed. The preferred rodent species is the rat and the preferred non-rodent species is the rabbit.

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

(iii) **Sex.** Nulliparous female animals should be used at each dose level. Animals should be mated with males of the same species and strain, avoiding the mating of siblings, if parentage is known. Day 0 in the test is the day on which a vaginal plug and/or sperm are observed in the rodent or that insemination is performed or observed in the rabbit.

(iv) **Animal care.** Animal care and housing should be in accordance with the recommendations contained in the DHHS/PHS NIH Publication No. 86–23, 1985, *Guidelines for the Care and Housing of Laboratory Animals*, or other appropriate guidelines.

(v) **Number of animals.** Each test and control group should contain a sufficient number of animals to yield approximately 20 animals with implantation sites at necropsy.

(2) **Administration of test and control substances—(i) Dose levels and dose selection.** (A) At least three-dose levels and a concurrent control should be used. Healthy animals should be randomly assigned to the control and treatment groups, in a manner which results in comparable mean body weight values among all groups. The dose levels should be spaced to produce a gradation of toxic effects. Unless limited by the physical/chemical nature or biological properties of the test substance, the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity but not death or severe suffering. In the case of maternal mortality, this should not be more than approximately 10 percent. The intermediate dose levels should produce minimal observable toxic effects. The lowest dose level should not produce any evidence of either maternal or developmental toxicity (i.e., the no-observed-adverse-effect level, NOAEL) or should be at or near the limit of detection for the most sensitive endpoint. Two- or four-fold intervals are frequently optimal for spacing the dose levels, and the addition of a fourth test group is often preferable to using very large intervals (e.g., more than a factor of 10) between dosages.

(B) It is desirable that additional information on metabolism and pharmacokinetics of the test substance be available to demonstrate the adequacy of the dosing regimen. This information should be available prior to testing.

(C) The highest dose tested need not exceed 1,000 mg/kg/day by oral or dermal administration, or 2 mg/L (or the maximum attainable concentration) by inhalation, unless potential human exposure data indicate the need for higher doses. If a test performed at the limit dose level, using the procedures described for this study, produces no observable toxicity and if an effect would not be expected based upon data from structurally related compounds, then a full study using three-dose levels may not be considered necessary.

(ii) **Control group.** (A) A concurrent control group should be used. This group should be a sham-treated control group or a vehicle-control group if a vehicle is used in administering the test substance.

(B) The vehicle control group should receive the vehicle in the highest volume used.

(C) If a vehicle or other additive is used to facilitate dosing, consideration should be given to the following characteristics: Effects on the absorption, distribution, metabolism, or retention of the test substance; effects on the chemical properties of the test substance which may alter its toxic

characteristics; and effects on the food or water consumption or the nutritional status of the animals.

(iii) **Route of administration.** (A) The test substance or vehicle is usually administered orally by intubation.

(B) If another route of administration is used, for example, when the route of administration is based upon the principal route of potential human exposure, the tester should provide justification and reasoning for its selection, and appropriate modifications may be necessary. Further information on dermal or inhalation exposure is provided under paragraphs (h)(12), (h)(28), and (h)(29) of this guideline. Care should be taken to minimize stress on the maternal animals. For materials administered by inhalation, whole-body exposure is preferable to nose-only exposure due to the stress of restraint required for nose-only exposure.

(C) The test substance should be administered at approximately the same time each day.

(D) When administered by gavage or dermal application, the dose to each animal should be based on the most recent individual body weight determination.

(iv) **Dosing schedule.** At minimum, the test substance should be administered daily from implantation to the day before cesarean section on the day prior to the expected day of parturition. Alternatively, if preliminary studies do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from fertilization to approximately 1 day prior to the expected day of termination.

(f) **Observation of animals—(1) Maternal.** (i) Each animal should be observed at least once daily, considering the peak period of anticipated effects after dosing. Mortality, moribundity, pertinent behavioral changes, and all signs of overt toxicity should be recorded at this cageside observation. In addition, thorough physical examinations should be conducted at the same time maternal body weights are recorded.

(ii) Animals should be weighed on day 0, at termination, and at least at 3-day intervals during the dosing period.

(iii) Food consumption should be recorded on at least 3-day intervals, preferably on days when body weights are recorded.

(iv) Termination schedule. (A) Females should be terminated immediately prior to the expected day of delivery.

(B) Females showing signs of abortion or premature delivery prior to scheduled termination should be killed and subjected to a thorough macroscopic examination.

(v) Gross necropsy. At the time of termination or death during the study, the dam should be examined macroscopically for any structural abnormalities or pathological changes which may have influenced the pregnancy. Evaluation of the dams during cesarean section and subsequent fetal analyses should be conducted without knowledge of treatment group in order to minimize bias.

(vi) Examination of uterine contents. (A) Immediately after termination or as soon as possible after death, the uteri should be removed and the pregnancy status of the animals ascertained. Uteri that appear non-gravid should be further examined (e.g. by ammonium sulfide staining) to confirm the nonpregnant status.

(B) Each gravid uterus (with cervix) should be weighed. Gravid uterine weights should not be obtained from dead animals if autolysis or decomposition has occurred.

(C) The number of corpora lutea should be determined for pregnant animals.

(D) The uterine contents should be examined for embryonic or fetal deaths and the number of viable fetuses. The degree of resorption should be described in order to help estimate the relative time of death of the conceptus.

(2) **Fetal.** (i) The sex and body weight of each fetus should be determined.

(ii) Each fetus should be examined for external anomalies.

(iii) Fetuses should be examined for skeletal and soft tissue anomalies (e.g. variations and malformations or other categories of anomalies as defined by the performing laboratory).

(A) For rodents, approximately one-half of each litter should be prepared by standard techniques and examined for skeletal alterations, preferably bone and cartilage. The remainder should be prepared and examined for soft tissue anomalies, using appropriate serial sectioning or gross dissection techniques. It is also acceptable to examine all fetuses by careful dissection for soft tissue anomalies followed by an examination for skeletal anomalies.

(B) For rabbits, all fetuses should be examined for both soft tissue and skeletal alterations. The bodies of these fetuses should be evaluated by careful dissection for soft-tissue anomalies, followed by preparation and examination for skeletal anomalies. An adequate evaluation of the internal structures of the head, including the eyes, brain, nasal passages, and tongue, should be conducted for at least half of the fetuses.

(g) **Data and reporting**—(1) **Treatment of results.** Data should be reported individually and summarized in tabular form, showing for each test group the types of change and the number of dams, fetuses, and litters displaying each type of change.

(2) **Evaluation of study results.** The following should be provided:

(i) Maternal and fetal test results, including an evaluation of the relationship, or lack thereof, between the exposure of the animals to the test substance and the incidence and severity of all findings.

(ii) Criteria used for categorizing fetal external, soft tissue, and skeletal anomalies.

(iii) When appropriate, historical control data to enhance interpretation of study results. Historical data (on litter incidence and fetal incidence within litter), when used, should be compiled, presented, and analyzed in an appropriate and relevant manner. In order to justify its use as an analytical tool, information such as the dates of study conduct, the strain and source of the animals, and the vehicle and route of administration should be included.

(iv) Statistical analysis of the study findings should include sufficient information on the method of analysis, so that an independent reviewer/statistician can reevaluate and reconstruct the analysis. In the evaluation of study data, the litter should be considered the basic unit of analysis.

(v) In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailability of the test substance should be considered.

(3) **Test report.** In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, and 40 CFR part 160, subpart J, the following specific information should be reported. Both individual and summary data should be presented.

(i) Species and strain.

(ii) Maternal toxic response data by dose, including but not limited to:

(A) The number of animals at the start of the test, the number of animals surviving, the number pregnant, and the number aborting.

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

(C) Day of observation of each abnormal clinical sign and its subsequent course.

(D) Body weight and body weight change data, including body weight change adjusted for gravid uterine weight.

(E) Food consumption and, if applicable, water consumption data.

(F) Necropsy findings, including gravid uterine weight.

(iii) Developmental endpoints by dose for litters with implants, including:

(A) Corpora lutea counts.

(B) Implantation data, number and percent of live and dead fetuses, and resorptions (early and late).

(C) Pre- and postimplantation loss calculations.

(iv) Developmental endpoints by dose for litters with live fetuses, including:

(A) Number and percent of live offspring.

(B) Sex ratio.

(C) Fetal body weight data, preferably by sex and with sexes combined.

(D) External, soft tissue, and skeletal malformation and variation data. The total number and percent of fetuses and litters with any external, soft tissue, or skeletal alteration, as well as the types and incidences of individual anomalies, should be reported.

(v) The numbers used in calculating all percentages or indices.

(vi) Adequate statistical treatment of results.

(vii) A copy of the study protocol and any amendments should be included.

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

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