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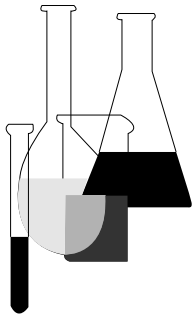
Health Effects Test Guidelines OPPTS 870.3500 Preliminary Developmental Toxicity Screen



“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.

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OPPTS 870.3500 Preliminary developmental toxicity screen.

(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 798.4420 Preliminary Developmental Toxicity Screen.

(b) **Purpose.** The **in vivo** developmental toxicity assay is designed to assess the potential of agents to induce toxic effects in the conceptus after administration to the mother during pregnancy. This test should be used only to prioritize environmental agents for testing by more rigorous standard protocols.

(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 definition also applies to this test guideline.

Developmental toxicity is the capability of an agent to induce in utero death, structural or functional abnormalities, or growth retardation after contact with the pregnant animal.

(d) **Principle of the test method.** The test substance is administered to pregnant animals during a significant portion of the major period of organogenesis. A single dose level is administered. This dose level should be high enough to elicit significant maternal toxicity. The dams are allowed to give birth and the neonates are counted and weighed on day–1 and day–3 postpartum (day–1 is the day after birth). The underlying hypothesis for this assay is that most prenatal insults will manifest themselves as reduced viability and/or growth during the postnatal period.

(e) **Test procedures—**(1) **Animal selection—**(i) **Species and strain.** Testing must be performed in a mammalian species and strain which will allow human handling of newborn pups without cannibalization or abandonment. The preferred species would be either rat or mouse, and of necessity, a strain that does not exhibit the behavior referred to above. The strain should be commonly used and should not have low fecundity.

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

(iii) **Sex.** Pregnant female animals should be used.

(iv) **Number of animals.** At least 30 bred animals should be used for each compound. The objective is to ensure that sufficient litters 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 an untreated or sham treated control group, or, if a vehicle is used in administering the test substance, a vehicle control group. Except for treatment with the test substance, animals in the control group(s) should be handled in an identical manner to test group animals.

(3) **Dose levels and dose selection.** (i) A single dose level with a concurrent control and, when appropriate, a vehicle control, should be used.

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

(iii) To select the appropriate dose levels, a pilot or trial study may be advisable. It is not always necessary to carry out a trial study in pregnant animals. Comparison of the results from a trial study in non-pregnant, and the main study in pregnant animals will demonstrate whether or not the test substance is more toxic in pregnant animals.

(iv) Unless limited by the physical/chemical nature or biological properties of the substance, the dose level used should be high enough to cause overt maternal toxicity as evidenced by significant death, weight loss or other adverse clinical manifestations, or 1 gm/kg, if lower dose levels fail to induce maternal toxicity.

(4) **Observation period.** Day–0 in the test is the day in which a vaginal plug and/or sperm are observed. The dose period should encompass a significant portion of the period of major organogenesis. This may be taken as day–7 to day–11 for rat and mouse. When there is evidence of rapid clearance it may be advisable to extend the dosing period for 2 days to cover the critical period of palatal closure.

(5) **Administration of test substance.** The test substance or vehicle is usually administered orally, by intubation unless the chemical or physical characteristics of the test substance or pattern of human exposure suggest a more appropriate route of administration. The test substance should be administered at approximately the same time each day.

(6) **Exposure conditions.** The female test animals are treated with the test substance daily throughout the appropriate treatment period. When given by gavage, the dose may be based on the weight of the females at the start of substance administration, or, alternatively, in view of the rapid weight gain which takes place during pregnancy, the animals may be weighed periodically and the dosage based on the most recent weight determination.

(7) **Observation of pregnant animals.** (i) A gross examination of the dams should be made at least once prior to parturition.

(ii) Pregnant animals should be weighed the day prior to the beginning of treatment, and that day on which treatment ends.

(iii) Cage-side observations should include, but not be limited to: change in skin and fur, eye and mucous membranes, as well as respiratory, autonomic and central nervous systems, somatomotor activity and behavioral pattern.

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

(v) During the dosing period females that die or are sacrificed because they are moribund should be examined for signs of pregnancy and details of the conditions of the uterus and/or its contents recorded. Animals that have not delivered 2 days after expected date of parturition should be sacrificed and similar examinations made.

(8) **Observation of dams after birth.** Dams should be observed for signs of overt toxicity during the postpartum period at the same time neonatal examinations are being made.

(9) **Neonatal examinations.** (i) Dams are allowed to give birth and the litters are examined for gross anomalies and presence of milk, counted, and weighed on postpartum day–1 and day–3.

(ii) Dead pups should be subjected to a thorough external examination and gross soft tissue abnormalities noted.

(iii) For those compounds that induce only neonatal growth reduction it may be advisable to normalize litter size on postpartum day–3 (to approximately 4 females and 4 males) and leave them with the dam through weaning. This procedure will determine if the growth reduction is transient or if it represents a permanent functional alteration.

(f) **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 maternal weight during the treatment period, the average number of live neonates on day–1 and day–3, the average neonatal weight on day–1 and day–3, and the average weight gained during that period.

(2) **Evaluation of results.** The findings of this bioassay should be evaluated in terms of the types of effects noted. All data analyses should compare treatment groups and their concurrent controls. Statistical treatment of the results should involve analysis of variance, and the number of live pups on day–1 and day–3 should be used as a covariate in the analyses of postnatal body weight so as to correct for differences in pup weights due to litter size. The number of animals going to term must be sufficiently large to allow for a reasonable detection of compound-induced deficiencies. Conditions which significantly reduce the number of dams

going to term (e.g. lack of pregnancy or compound-induced maternal death) should lead to a repeat of the study.

(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) Toxic response data.

(ii) Species and strain.

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

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

(v) Pregnancy data.

(vi) Litter data including number live and dead; and average litter weight on day–1 and day–3 postpartum.

(vii) Necropsy data on dead pups.

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

(1) Chernoff, N. and Kavlock, R. An in vivo teratology screen utilizing pregnant mice, *Journal of Toxicology and Environmental Health* 10:541–550 (1982).

(2) Doe, J. E. et al. Comparative aspects of the reproductive toxicology by inhalation in rats of ethylene glycol monomethyl ether and propylene glycol monomethyl ether. *Toxicology and Applied Pharmacology* 69:43–47 (1983).