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§ 799.9370 TSCA prenatal developmental toxicity.

(a) *Scope* This section is intended to meet the testing requirements under section 4 of TSCA. This guideline for developmental toxicity testing is designed to provide general information concerning the effects of exposure on the pregnant test animal and 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.

(b) *Source.* The source material used in developing this TSCA test guideline is the OPPTS harmonized test guideline 870.3700 (February 1996 Public Draft). This source is available at the address in paragraph (h) of this section.

(c) *Good laboratory practice standards.* The study shall be conducted in compliance with 40 CFR Part 792—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 shall be used.

(iii) *Sex.* Nulliparous female animals shall 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) *Number of animals.* Each test and control group shall 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 shall be used. Healthy animals shall 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 shall 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%. 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 shall be used. This group shall 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 shall provide justification and reasoning for its selection, and appropriate modifications may be necessary. 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 shall be administered at approximately the same time each day.

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

(iv) *Dosing schedule.* At minimum, the test substance shall 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 shall 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 shall be recorded at this cageside observation. In addition, thorough physical examinations shall be conducted at the same time maternal body weights are recorded.

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

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

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

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

(v) At the time of termination or death during the study, the dam shall 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) (A) Immediately after termination or as soon as possible after death, the uteri shall be removed and the pregnancy status of the animals ascertained. Uteri that appear nongravid shall be further examined (e.g. by ammonium sulfide staining) to confirm the nonpregnant status.

(B) Each gravid uterus (with cervix) shall be weighed. Gravid uterine weights should not be obtained from

dead animals if autolysis or decomposition has occurred.

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

(D) The uterine contents shall be examined for embryonic or fetal deaths and the number of viable fetuses. The degree of resorption shall 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 shall be determined.

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

(iii) Fetuses shall 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 shall be prepared by standard techniques and examined for skeletal alterations, preferably bone and cartilage. The remainder shall 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 shall 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 shall 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 shall 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, the following specific information shall 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.* For additional background information on this test guideline, the following references should be consulted. These references are available for inspection at the TSCA Nonconfidential Information Center, Rm. NE-B607, Environmental Protection Agency, 401 M St., SW., Washington, DC, 12 noon to 4 p.m., Monday through Friday, except legal holidays.

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§ 799.9380 TSCA reproduction and fertility effects.

(a) *Scope.* This section is intended to meet the testing requirements under section 4 of the TSCA. This section is for two-generation reproduction testing and is designed to provide general information concerning the effects of a test substance on the integrity and performance of the male and female reproductive systems, including gonadal function, the estrous cycle, mating behavior, conception, gestation, parturition, lactation, and weaning, and on the growth and development of the offspring. The study may also provide information about the effects of the test substance on neonatal morbidity, mortality, target organs in the offspring, and preliminary data on prenatal and postnatal developmental toxicity and serve as a guide for subsequent tests. Additionally, since the study design includes *in utero* as well as postnatal exposure, this study provides the opportunity to examine the susceptibility of the immature/neonatal animal.

(b) *Source.* The source material used in developing this TSCA test guideline is the OPPTS harmonized test guideline 870.3800 (February 1996 Public Draft). This source is available at the address in paragraph (g) of this section.

(c) *Good laboratory practice standards.* The study shall be conducted in compliance with 40 CFR part 792—Good Laboratory Practice Standards.

(d) *Principle of the test method.* The test substance is administered to parental (P) animals prior to and during their mating, during the resultant pregnancies, and through the weaning of their F1 offspring. The substance is then administered to selected F1 offspring during their growth into adulthood, mating, and production of an F2 generation, until the F2 generation is weaned.

(e) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* The rat is the most commonly used species for testing. If another mammalian species is used, the tester shall provide justification/reasoning for its selection, and appropriate modifications will be necessary. Healthy parental animals, which have been acclimated to laboratory conditions for at least 5 days and have not been subjected to previous experimental procedures, should be used. Strains of low fecundity shall not be used.

(ii) *Age.* Parental (P) animals shall be 5 to 9 weeks old at the start of dosing. The animals of all test groups should be of uniform weight, age, and parity as nearly as practicable, and should be representative of the species and strain under study.

(iii) *Sex.* (A) For an adequate assessment of fertility, both males and females shall be studied.