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 shall 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 shall be described.

(ii) *Exposure data.* These shall be tabulated and presented with mean values and a measure of variability (e.g., standard deviation) and shall 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 sketetal defects, resorptions).

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Department of Health and Welfare. *The Testing of Chemicals for Carcinogenicity, Mutagenicity and Teratogenicity*. Minister of Health and Welfare (Canada: Department of Health and Welfare, 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. (Geneva: World Health Organization, 1967).

[50 FR 39397, Sept. 27, 1985, as amended at 52 FR 19076, May 20, 1987; 52 FR 26150, July 13, 1987; 54 FR 21064, May 16, 1989]

§ 798.4700 Reproduction and fertility effects.

(a) *Purpose.* This guideline for two-generation reproduction testing is designed to provide general information concerning the effects of a test substance on gonadal function, conception, parturition, and the growth and development of the offspring. The study may also provide information about the effects of the test substance on neonatal morbidity, mortality, and preliminary data on teratogenesis and serve as a guide for subsequent tests.

(b) *Principle of the test method.* The test substance is administered to parental (P) animals prior to their mating, during the resultant pregnancies, and through the weaning of their F_1 offspring. The substance is then administered to selected F_1 offspring during their growth into adulthood, mating, and production of an F_2 generation, up until the F_2 generation is weaned.

(c) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* The rat is the preferred species. If another mammalian species is used, the tester shall provide justification/reasoning for its

selection. Strains with low fecundity shall not be used.

(ii) *Age.* Parental (P) animals shall be about 5 to 8 weeks old at the start of dosing.

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

(B) The females shall be nulliparous and non-pregnant.

(iv) *Number of animals.* Each test and control group shall contain at least 20 males and a sufficient number of females to yield at least 20 pregnant females at or near term.

(2) *Control groups.* (i) A concurrent control group shall be used. This group shall be an untreated or sham treated control group or if a vehicle is used in administering the test substance, a vehicle control group.

(ii) If a vehicle is used in administering the test substance, the control group shall receive the vehicle in the highest volume used.

(iii) If a vehicle or other additive is used to facilitate dosing, it shall not interfere significantly with absorption of the test substance or produce toxic effects.

(3) *Dose levels and dose selection.* (i) At least three dose levels and a concurrent control shall be used.

(ii) The highest dose level should induce toxicity but not high levels of mortality in the parental (P) animals.

(iii) The lowest dose level should not produce any grossly observable evidence of toxicity.

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

(4) *Exposure conditions.* The animals should be dosed with the test substance, ideally, on a 7 days per week basis.

(i) Dosing, mating, delivery, and sacrifice schedule.

(A) Daily dosing of the parental (P) males and females shall begin when they are 5 to 8 weeks old. For both sexes, dosing shall be continued for at least 10 weeks before the mating period.

(B) Dosing of P males shall continue through the 3 week mating period. At the end of the mating period, P males may be sacrificed and examined, or may be retained for possible production of a second litter. If these animals are retained for a second litter, dosing shall be continued. Dosing of the F_1 males saved for mating shall continue from the time they are weaned through the period they are mated with the F_1 females (11 weeks). F_1 males may be sacrificed after the F_1 mating period.

(C) Daily dosing of the P females shall continue through the three week mating period, pregnancy, and to the weaning of the F_1 offspring. Dosing of the F_1 females saved for mating shall continue from the time they are weaned, through the period they are mated with the F_1 males (11 weeks from the time of weaning) pregnancy, and to the weaning of the F_2 offspring.

(ii) All animals are sacrificed as scheduled.

(A) All P males should be sacrificed at the end of the 3-week mating period, or may be retained for possible production of a second litter. If these animals are retained for a second litter, dosing shall be continued.

(B) F_1 males selected for mating should be sacrificed at the end of the three week period of the F_1 mating.

(C) F_1 males and females not selected for mating should be sacrified when weaned.

(D) The P females should be sacrificed upon weaning of their F_1 offspring.

(E) F_1 dams and their F_2 offspring are sacrificed when the offspring are weaned.

(5) *Administration of the test substance*—(i) *Oral studies.* (A) It is recommended that the test substance be administered in the diet or drinking water.

(B) If administered by gavage or capsule, the dosage administered to each animal prior to mating shall be based on the individual animal's body weight and adjusted weekly. During pregnancy the dosage shall be based on the body weight at day 0 and 6 of pregnancy.

(ii) If another route of administration is used, the tester should provide justification and reasoning for its selection.

(6) *Mating procedure*—(i) *Parental.* (A) For each mating, each female shall be

placed with a single male from the same dose level until pregnancy occurs or 1 week has elapsed. If mating has not occurred after 1 week, the female shall be placed with a different male. Paired matings should be clearly identified.

(B) Those pairs that fail to mate should be evaluated to determine the cause of the apparent infertility. This may involve such procedures as additional opportunities to mate with proven fertile males or females, histological examination of the reproductive organs, and examination of the estrus or spermatogenic cycles.

(C) Each day, the females shall be examined for presence of sperm or vaginal plugs. Day 0 of pregnancy is defined as the day vaginal plugs or sperm are found.

(ii) *F_1* cross. (A) For mating the F_1 offspring, one male and one female are randomly selected at weaning from each litter for cross mating with another pup of the same dose level but different litter, to produce the F_2 generation.

(B) F_1 males and females not selected for mating are sacrificed upon weaning.

(iii) *Special housing.* After evidence of copulation, pregnant animals shall be caged separately in delivery or maternity cages. Pregnant animals shall be provided with nesting materials when parturition is near.

(iv) *Standardization of litter sizes.* (A) On day 4 after birth, the size of each litter should be adjusted by eliminating extra pups by random selection to yield, as nearly as possible, 4 males and 4 females per litter.

(B) Whenever the number of male or female pups prevents having 4 of each sex per litter, partial adjustment (for example, 5 males and 3 females) is permitted. Adjustments are not appropriate for litters of less than 8 pups.

(C) Elimination of runts only is not appropriate.

(D) Adjustments of the F_2 litters is conducted in the same manner.

(7) *Observation of animals.* (i) A gross examination shall be made at least once each day. Pertinent behavioral changes, signs of difficult or prolonged parturition, and all signs of toxicity, including mortality, shall be recorded. These observations shall be reported for each individual animal. Food consumption for all animals shall be monitored weekly except during the mating period.

(ii) The duration of gestation shall be calculated from day 0 of pregnancy.

(iii) Each litter should be examined as soon as possible after delivery for the number of pups, stillbirths, live births, sex, and the presence of gross anomalies. Live pups should be counted and litters weighed at birth or soon thereafter, and on days 4, 7, 14, and 21 after parturition.

(iv) Physical or behavioral abnormalities observed in the dams of offspring shall be recorded.

(v) P males and females shall be weighed on the first day of dosing and weekly thereafter. F_1 litters shall be weighed at birth, or soon thereafter, and on days 4, 7, 14, and 21. In all cases, litter weights shall be calculated from the weights of the individual pups.

(8) *Gross necropsy.* (i) A complete gross examination shall be performed on all adult animals, including those which died during the experiment or were killed in moribund conditions.

(ii) Special attention shall be directed to the organs of the reproductive system.

(iii) The following organs and tissues, or representative samples thereof, shall be preserved in a suitable medium for possible future histopathological examination: Vagina; uterus; ovaries; testes; epididymides; seminal vesicles; prostate, pituitary gland; and, target organ(s) when previously identified of all P and F_1 animals selected for mating.

(9) *Histopathology.* Except if carried out in other studies of comparable duration and dose levels the following histopathology shall be performed:

(i) Full histopathology on the organs listed above for all high dose, and control P_1 and F_1 animals selected for mating.

(ii) Organs demonstrating pathology in these animals shall then be examined in animals from the other dose groups.

(iii) Microscopic examination shall be made of all tissues showing gross pathological changes.

(d) *Data and reporting*—(1) *Treatment of results.* Data shall be summarized in

tabular form, showing for each test group the number of animals at the start of the test, the number of animals pregnant, the types of change and the percentage of animals displaying each type of change.

(2) *Evaluation of study results.* (i) An evaluation of test results, including the statistical analysis, based on the clinical findings, the gross necropsy findings, and the microscopic results shall be made and supplied. This should include an evaluation of the relationship, or lack thereof, between the animals' exposure to the test substance and the incidence and severity of all abnormalities.

(ii) 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:

(i) Toxic response data by sex and dose, including fertility, gestation, viability and lactation indices, and length of gestation.

(ii) Species and strain.

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

(iv) Toxic or other effects on reproduction, offspring, or postnatal growth.

(v) Date of observation of each abnormal sign and its subsequent course.

(vi) Body weight data for P, F_1, and F_2 animals.

(vii) Necropsy findings.

(viii) Detailed description of all histopathological findings.

(ix) Statistical treatment of results where appropriate.

(e) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Clermont, Y., Perry, B. ''Quantitative Study of the Cell Population of the Seminiferous Tubules in Immature Rats,'' *American Journal of Anatomy*. 100:241–267 (1957).

(2) Goldenthal, E.I. *Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use.* Drug Review Branch, Division of Toxicological Evaluation, Bureau of Science, Food and Drug Administration, Washington, DC (1966).

(3) Hasegawa, T., Hayashi, M., Ebling, F.J.G., Henderson, I.W. *Fertility and Sterility*. (New York: American Elsevier Publishing Co., Inc., 1973).

(4) Oakberg, E.F. ''Duration of Spermatogenesis in the Mouse and Timing of Stages of the Cycle of the Seminiferous Epithelium,'' *American Journal of Anatomy*. 9:507–516 (1956).

(5) Roosen-Runge, E.C. ''The Process of Spermatogenesis in Mammals,'' *Biological Review*. 37:343–377 (1962).

[50 FR 39397, Sept. 27, 1985, as amended at 52 FR 19077, May 20, 1987]

§ 798.4900 Developmental toxicity study.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a chemical, determination of the potential developmental toxicity is important. The 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.

(b) *Definitions.* (1) 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.

(2) Dose is the amount of test substance administered. Dose is expressed as weight of test substance (g, mg) per unit weight of a test animal (e.g., mg/kg).

(3) No-observed-effect level is the maximum concentration in a test which produces no observed adverse effects. A no-observed-effect level is expressed in terms of weight of test substance given daily per unit weight of test animal (mg/kg)

(c) *Principle of the test method.* The test substance is administered in graduated doses for at least part of the pregnancy covering the major period of organogenesis, to several groups of pregnant experimental animals, one dose 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.