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

(E) Exposure data shall be tabulated and presented with mean values and a measure of variability (e.g., standard deviation) and include:

(*1*) Airflow rates through the inhalation equipment.

(*2*) Temperature and humidity of air.

(*3*) Actual (analytical or gravimetric) concentration in the breathing zone.

(*4*) Nominal concentration (total amount of test substance fed into the inhalation equipment divided by volume of air).

(*5*) Particle size distribution, calculated mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD).

(*6*) Explanation as to why the desired chamber concentration and/or particle size could not be achieved (if applicable) and the efforts taken to comply with this aspect of the section.

(iv) Test results information shall include:

(A) *Group animal data.* Tabulation of toxic response data by species, strain, sex and exposure level for:

(*1*) Number of animals exposed.

(*2*) Number of animals showing signs of toxicity.

(*3*) Number of animals dying.

(B) *Individual animal data.* Data should be presented as summary (group mean) as well as for individual animals.

(*1*) Time of death during the study or whether animals survived to termination.

(*2*) Time of observation of each abnormal sign and its subsequent course.

(*3*) Body weight data.

(*4*) Feed consumption data, when collected.

(*5*) Results of ophthalmological examination, when performed.

(*6*) Results of hematological tests performed. .

(*7*) Results of clinical chemistry tests performed.

(*8*) Results of urinalysis tests performed.

(*9*) Necropsy findings, including absolute and relative organ weight data.

(*10*) Detailed description of all histopathological findings.

(*11*) Statistical treatment of results, where appropriate.

(g) *Quality control.* A system shall be developed and maintained to assure and document adequate performance of laboratory staff and equipment. The study shall be conducted in compliance with 40 CFR part 792—Good Laboratory Practice Standards.

(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.

(1) Cage, J.C. Ed. Paget, G.E. *Experimental Inhalation Toxicology, Methods in Toxicology*. (F.A. Davis Co., Philadelphia, PA, 1970) pp. 258–277.

(2) Casarett, L.J. and Doull. Chapter 9. *Toxicology: The Basic Science of Poisons* (New York: Macmillan Publishing Co., Inc., 1975).

(3) U.S. Environmental Protection Agency, Office of Pesticide Programs, Health Effects Division. Interim policy for particle size and limit concentration issues in inhalation toxicity studies (February 1, 1994).

(4) MacFarland, H.N. Ed. Hayes, W.J. Vol. 7. *Respiratory Toxicology, Essays in Toxicology*. (Academic Press, New York, NY, 1976) pp. 121–154.

(5) Organisation for Economic Co-operation and Development. Guidelines for testing of chemicals, section 4-health effects, part 413. *Subchronic Inhalation Toxicity Studies* (Paris, 1981).

[62 FR 43824, Aug. 15, 1997, as amended at 64 FR 35077, June 30, 1999]

§ 799.9355 TSCA reproduction/developmental toxicity screening test.

(a) *Scope*—(1) *Applicability.* This section is intended to meet testing requirements of the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) *Source.* The source material used in developing this TSCA test guideline is the Office of Prevention, Pesticides, and Toxic Substances (OPPTS) harmonized test guideline 870.3550 (July 2000, final guidelines). This source is available at the address in paragraph (h) of this section.

(b) *Purpose.* (1) This guideline is designed to generate limited information concerning the effects of a test substance on male and female reproductive performance such as gonadal function, mating behavior, conception, development of the conceptus, and parturition. It is not an alternative to, nor does it replace, the existing comprehensive test standards in §§ 799.9370 and 799.9380.

(2) This screening test guideline can be used to provide initial information on possible effects on reproduction and/or development, either at an early stage of assessing the toxicological properties of chemicals, or on chemicals of high concern. It can also be used as part of a set of initial screening tests for existing chemicals for which little or no toxicological information is available, as a dose range finding study for more extensive reproduction/developmental studies, or when otherwise considered relevant.

(3) This test does not provide complete information on all aspects of reproduction and development. In particular, it offers only limited means of detecting postnatal manifestations of prenatal exposure, or effects that may be induced during postnatal exposure. Due (amongst other reasons) to the relatively small numbers of animals in the dose groups, the selectivity of the end points, and the short duration of the study, this method will not provide evidence for definite claims of no effects.

(c) *Definitions.* The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards apply to this section. The following definitions also apply to this section.

Dosage is a general term comprising of dose, its frequency and the duration of dosing.

Dose is the amount of test substance administered. Dose is expressed as weight (g, mg) as weight of test substance per unit weight of test animal (e.g., mg/kg), or as constant dietary concentration parts per million (ppm).

No-observed-effects level (NOEL) is the maximum dose used in a study which produces no adverse effects. The NOEL is expressed in terms of the weight of a test substance given daily per unit weight of test animal (milligrams per kilograms per day).

(d) *Principle of the test.* (1) The test substance is administered in graduated doses to several groups of males and females. Males should be dosed for a minimum of four weeks and up to and including the day before scheduled sacrifice (this includes a minimum of two weeks prior to mating, during the mating period and, approximately, two weeks post-mating). In view of the limited pre-mating dosing period in males, fertility may not be a particular sensitive indicator of testicular toxicity. Therefore, a detailed histological examination of the testes is essential. The combination of a pre-mating dosing period of two weeks and subsequent mating/fertility observations with an overall dosing period of at least four weeks, followed by detailed histopathology of the male gonads, is considered sufficient to enable detection of the majority of effects on male fertility and spermatogenesis.

(2) Females should be dosed throughout the study. This includes two weeks prior to mating (with the objective of covering at least two complete oestrous cycles), the variable time to conception, the duration of pregnancy and at least four days after delivery, up to and including the day before scheduled sacrifice.

(3) Duration of study, following acclimatization, is dependent on the female performance and is approximately 54 days, (at least 14 days premating, (up to) 14 days mating, 22 days gestation, 4 days lactation).

(4) During the period of administration, the animals are observed closely each day for signs of toxicity. Animals which die or are sacrificed during the test period are necropsied and, at the conclusion of the test, surviving animals are sacrificed and necropsied.

(e) *Description of the method*—(1) *Selection of animal species.* This test standard is designed for use with the rat. If other species are used, appropriate modifications will be necessary. Strains with low fecundity or well-known high incidence of developmental defects should not be used. Healthy virgin animals, not subjected to previous experimental procedures, should be

used. The test animals should be characterized as to species, strain, sex, weight and/or age. At the commencement of the study the weight variation of animals used should be minimal and not exceed 20% of the mean weight of each sex.

(2) *Housing and feeding conditions.* (i) The temperature in the experimental animal room should be 22 °C (±3°). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning, the aim should be 50–60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. The choice of diet may be influenced by the need to ensure a suitable admixture of a test substance when administered by this method.

(ii) Animals may be housed individually or be caged in small groups of the same sex; for group caging, no more than five animals should be housed per cage. Mating procedures should be carried out in cages suitable for the purpose. Pregnant females should be caged individually and provided with nesting materials.

(3) *Preparation of the animals.* Healthy young adult animals must be randomly assigned to the control and treatment groups. Cages should be arranged in such a way that possible effects due to cage placement are minimized. The animals must be uniquely identified and kept in their cages for at least five days prior to the start of the study to allow for acclimatization to the laboratory conditions.

(4) *Preparation of doses.* (i) It is recommended that the test substance be administered orally unless other routes of administration are considered more appropriate. When the oral route is selected, the test compound is usually administered by gavage; however, alternatively, test compounds may be administered via the diet or drinking water.

(ii) Where necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that, wherever possible, the use of an aqueous solution/suspension be considered first, followed by consideration of a solution/emulsion in oil (e.g., corn oil) and then by possible solution in other vehicles. For vehicles other than water the toxic characteristics of the vehicle must be known. The stability of the test substance in the vehicle should be determined.

(f) *Procedure*—(1) *Number and sex of animals.* It is recommended that each group be started with at least 10 animals of each sex. Except in the case of marked toxic effects, it is expected that this will provide at least 8 pregnant females per group which normally is the minimum acceptable number of pregnant females per group. The objective is to produce enough pregnancies and offspring to assure a meaningful evaluation of the potential of the substance to affect fertility, pregnancy, maternal and suckling behaviour, and growth and development of the F_1 offspring from conception to day 4 postpartum.

(2) *Dosage.* (i) Generally, at least three test groups and a control group should be used. Dose levels may be based on information from acute toxicity tests or on results from repeated dose studies. Except for treatment with the test substance, animals in the control group should be handled in an identical manner to the test group subjects. If a vehicle is used in administering the test substance, the control group should receive the vehicle in the highest volume used.

(ii) Dose levels should be selected taking into account any existing toxicity and (toxico-) kinetic data available for the test compound or related materials. The highest dose level should be chosen with the aim of inducing toxic effects but not death or severe suffering. Thereafter, a descending sequence of dose levels should be selected in order to demonstrate any dose response relationships and no adverse effects at the lowest dose level. Two to four fold intervals are frequently optimal for setting the descending dose levels and addition of a fourth test group is often preferable to using very large intervals (e.g., more than a factor of 10) between dosages.

(3) *Limit test.* If an oral study at one dose level of at least 1000 mg/kg body weight/day or, for dietary or drinking water administration, an equivalent percentage in the diet, or drinking

water using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using several dose levels may not be considered necessary. The limit test applies except when human exposure indicates the need for a higher oral dose level to be used. For other types of administration, such as inhalation or dermal application, the physical chemical properties of the test substance often may dictate the maximum attainable concentration.

(4) *Administration of doses.* (i) The animals must be dosed with the test substance daily for seven days a week. When the test substance is administered by gavage, this should be done in a single dose to the animals using a stomach tube or a suitable intubation cannula. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. The volume should not exceed 1 ml/100 g body weight, except in the case of aqueous solutions where 2 ml/100 g body weight may be used. Except for irritating substances which will normally reveal exacerbated effects with higher concentrations, variability in test volume should be minimized by adjusting the concentration to ensure a constant volume at all dose levels.

(ii) For substances administered via the diet or drinking water, it is important to ensure that the quantities of the test substance involved do not interfere with normal nutrition or water balance. When the test substance is administered in the diet either a constant dietary concentration (parts per million (ppm)) or a constant dose level in terms of the animals' body weight may be used; the alternative used must be specified. For a substance administered by gavage, the dose should be given at similar times each day, and adjusted at least weekly to maintain a constant dose level in terms of animal body weight.

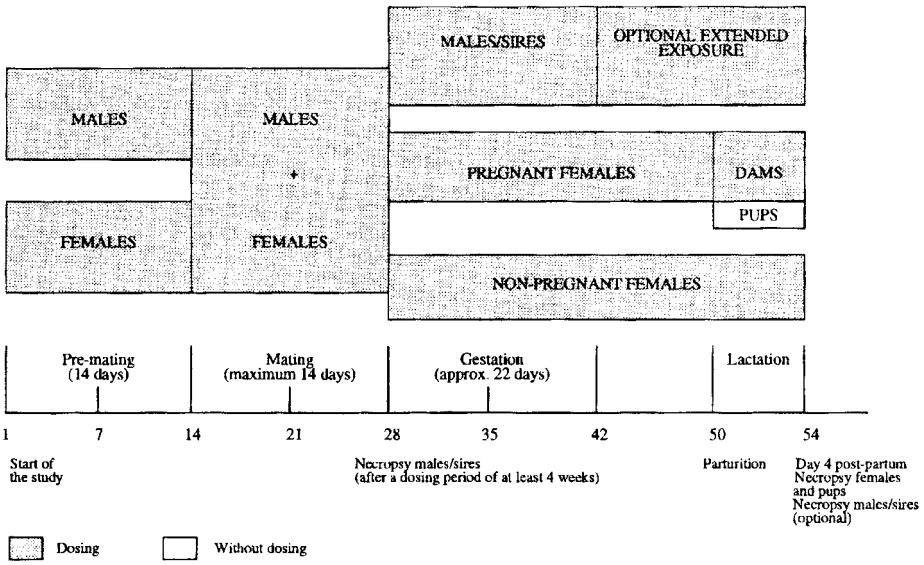
(5) *Experimental schedule.* (i) Dosing of both sexes should begin at least 2 weeks prior to mating, after they have been acclimatized for at least five days. The study should be scheduled in such a way that mating begins soon after the animals have attained full sexual maturity. This may vary slightly for different strains of rats in different laboratories, e.g., Sprague Dawley rats 10 weeks of age, Wistar rats about 12 weeks of age. Dams with offspring should be sacrificed on day 4 post-partum, or shortly thereafter. The day of birth (viz. when parturition is complete) is defined as day 0 post-partum. Females showing no-evidence of copulation are sacrificed 24–26 days after the last day of the mating period. Dosing is continued in both sexes during the mating period. Males should further be dosed after the mating period at least until the minimum total dosing period of 28 days has been completed. They are then sacrificed, or, alternatively, are retained and continued to be dosed for the possible conduction of a second mating if considered appropriate.

(ii) Daily dosing of the parental females should continue throughout pregnancy and at least up to, and including, day 3 post-partum or the day before sacrifice. For studies where the test substance is administered by inhalation or by the dermal route, dosing should be continued at least up to, and including, day 19 of gestation.

(iii) The experimental schedule is given in the following figure 1.

FIGURE 1

DIAGRAM OF THE EXPERIMENTAL SCHEDULE INDICATING THE MAXIMUM STUDY DURATION, BASED ON A FULL 14-DAY MATING PERIOD



(6) *Mating procedure.* Normally, 1:1 (one male to one female) matings should be used in this study. Exceptions can arise in the case of occasional deaths of males. The female should be placed with the same male until pregnancy occurs or two weeks have elapsed. Each morning the females should be examined for the presence of sperm or a vaginal plug. Day 0 of pregnancy is defined as the day a vaginal plug or sperm is found.

(7) *Observations.* (i) Throughout the test period, general clinical observations should be made at least once a day, and more frequently when signs of toxicity are observed. They should be made preferably at the same time(s) each day, considering the peak period of anticipated effects after dosing. Pertinent behavioural changes, signs of difficult or prolonged parturition and all signs of toxicity, including mortality, should be recorded. These records should include time of onset, degree and duration of toxicity signs.

(ii) The duration of gestation should be recorded and is calculated from day 0 of pregnancy. Each litter should be examined as soon as possible after delivery to establish the number and sex of pups, stillbirths, live births, runts (pups that are significantly smaller than corresponding control pups) and the presence of gross abnormalities.

(iii) Live pups should be counted and sexed and litters weighed within 24 hours of parturition (day 1) and on day 4 post-partum. In addition to the observations on parent animals, described by paragraph (f)(7) of this section, any abnormal behaviour of the offspring should be recorded.

(8) *Body weight and food/water consumption.* (i) Males and females should be individually weighed on the first day of dosing, at least weekly thereafter, and at termination. During pregnancy, females should be weighed on days 0, 7, 14 and 20 and within 24 hours of parturition (day 1) and day 4 post-partum.

(ii) During pre-mating, pregnancy and lactation, food consumption should be measured at least weekly. The measurement of food consumption during mating is optional. Water consumption during these periods should also be

measured when the test substance is administered via drinking water.

(9) *Pathology*—(i) *Gross necropsy*. (A) At the time of sacrifice or death during the study, the adult animals should be examined macroscopically for any abnormalities or pathological changes. Special attention should be paid to the organs of the reproductive system. The number of implantation sites should be recorded. Corpora lutea should be counted.

(B) The testes and epididymides of all male adult animals should be weighed.

(C) Dead pups and pups sacrificed at day 4 post-partum, or shortly thereafter, should, at least, be carefully examined externally for gross abnormalities.

(D) The ovaries, testes, epididymides, accessory sex organs and all organs showing macroscopic lesions of all adult animals should be preserved. Formalin fixation is not recommended for routine examination of testes and epididymides. An acceptable method is the use of Bouin's fixative for these tissues.

(ii) *Histopathology*. (A) Detailed histological examination should be performed on the ovaries, testes and epididymides of the animals of the highest dose group and the control group. The other preserved organs may be examined when necessary. Examinations should be extended to the animals of other dosage groups when changes are seen in the highest dose group.

(B) Detailed testicular histopathological examination (e.g., using Bouin's fixative, paraffin embedding and transverse sections of 4-5 ±m thickness) should be conducted with special emphasis on stages of spermatogenesis and histopathology interstitial testicular cell structure. The evaluation should identify treatment-related effects such as retained spermatids, missing germ cell layers or types, multinucleated giant cells or sloughing of spermatogenic cells into the lumen (the specifications for the evaluation are discussed in paragraph (g)(2) of this section). Examination of the intact epididymis should include the caput, corpus, and cauda, which can be accomplished by evaluation of a longitudinal section. The epididymis should be evaluated for leukocyte infiltration, change in prevalence of cell types, aberrant cell types, and phagocytosis of sperm. PAS and hematoxylin staining may be used for examination of the male reproductive organs. Histopathological examination of the ovary should detect qualitative depletion of the primordial follicle population.

(g) *Data and reporting*—(1) *Data*. Individual animal data should be provided. Additionally, all data should be summarised in tabular form, showing for each test group the number of animals at the start of the test, the number of animals found dead during the test or sacrificed for humane reasons, the time of any death or humane sacrifice, the number of fertile animals, the number of pregnant females, the number of animals showing signs of toxicity, a description of the signs of toxicity observed, including time of onset, duration, and severity of any toxic effects, the types of histopathological changes, and all relevant litter data.

(2) *Evaluation of results.* (i) The findings of this toxicity study should be evaluated in terms of the observed effects, necropsy and microscopic findings. This evaluation must include the relationship between the dose of the test substance and the presence or absence, incidence and severity of abnormalities, including gross lesions, identified target organs, infertility, clinical abnormalities, affected reproductive and litter performance, body weight changes, effects on mortality and any other toxic effects.

(ii) Because of the short period of treatment of the male, the histopathology of the testis and epididymus must be considered along with the fertility data, when assessing male reproductive effects.

(iii) Due to the limited dimensions of the study, statistical analysis in the form of tests for ''significance'' are of limited value for many endpoints, especially reproductive endpoints. If statistical analyses are used then the method chosen should be appropriate for the distribution of the variable examined, and be selected prior to the start of the study. Because of the small group size, the use of historic control data (e.g.,

for litter size), where available, may also be useful as an aid to the interpretation of the study.

(3) *Test report.* The test report must include the following information:

(i) Test substance:

(A) Physical nature and, where relevant, physicochemical properties.

(B) Identification data.

(ii) Vehicle (if appropriate): Justification for choice of vehicle if other than water.

(iii) Test animals:

(A) Species/strain used.

(B) Number, age and sex of animals.

(C) Source, housing conditions, diet, etc.

(D) Individual weights of animals at the start of the test.

(iv) Test conditions:

(A) Rationale for dose level selection.

(B) Details of test substance formulation/diet preparation, achieved concentrations, stability and homogeneity of the preparation.

(C) Details of the administration of the test substance.

(D) Conversion from diet/drinking water test substance concentration (parts per million (ppm)) to the actual dose (mg/kg body weight/day), if applicable.

(E) Details of food and water quality.

(v) Results (toxic response data by sex and dose):

(A) Time of death during the study or whether animals survived to termination.

(B) Nature, severity and duration of clinical observations (whether reversible or not).

(C) Body weight/body weight change data.

(D) Food consumption and water consumption, if applicable.

(E) Effects on reproduction, including information on mating/precoital interval, fertility, fecundity and gestation duration.

(F) Effects on offspring, including number of pups born (live and dead), sex ratio, postnatal growth (pup weights) and survival (litter size), gross abnormalities and clinical observations during lactation.

(G) Body weight at termination and organ weight data for the parental animals.

(H) Necropsy data, including number of implantations and number of corpora lutea.

(I) Calculations of pre- and postimplantation loss.

(J) Detailed description of histopathological findings.

(K) Statistical treatment of results, where appropriate.

(vi) Discussion of results.

(vii) Conclusions.

(4) *Interpretation of results.* The study will provide evaluations of reproduction/developmental toxicity associated with administration of repeated doses. It could provide an indication of the need to conduct further investigations and provides guidance in the design of subsequent studies.

(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.

(1) OECD (1995). Reproduction/Developmental Toxicity Screening Test, OECD 421, OECD Guidelines for Testing of Chemicals.

(2) [Reserved]

[65 FR 78789, Dec. 15, 2000]

§ 799.9365 TSCA combined repeated dose toxicity study with the reproduction/developmental toxicity screening test.

(a) *Scope*—(1) *Applicability.* This section is intended to meet testing requirements of the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) *Source.* The source material used in developing this TSCA test guideline is the Office of Prevention, Pesticides and Toxic Substances (OPPTS) harmonized test guideline 870.3650 (July 2000, final guidelines). This source is available at the address in paragraph (h) of this section.

(b) *Purpose.* (1) This screening test provides limited information on systemic toxicity, neurotoxicity, and/or immunotoxicity following repeated exposure over a limited time period. In addition, it can be used to provide initial information on possible effects on