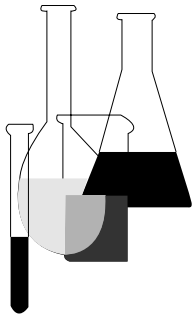
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–98–208
August 1998

Health Effects Test Guidelines OPPTS 870.3800 Reproduction and Fertility Effects



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 870.3800 Reproduction and fertility effects.

(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 798.4700, OPP guideline 83–4, and OECD guideline 416.

(b) **Purpose.** This guideline for two-generation reproduction testing 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. For further information on functional deficiencies and developmental effects, additional study segments can be incorporated into the protocol, utilizing the guidelines for developmental toxicity or developmental neurotoxicity.

(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 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 should 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 should not be used.

(ii) **Age.** Parental (P) animals should 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 should be studied.

(B) The females should be nulliparous and nonpregnant.

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

(v) **Number of animals.** Each control group should contain a sufficient number of mating pairs to yield approximately 20 pregnant females. Each test group should contain a similar number of mating pairs.

(vi) **Identification of animals.** Each animal should be assigned a unique identification number. For the P generation, this should be done before dosing starts. For the F1 generation, this should be done for animals selected for mating; in addition, records indicating the litter of origin should be maintained for all selected F1 animals.

(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 reproductive and/or systemic toxicity but not death or severe suffering. In the case of parental 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 systemic or reproductive 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 should not exceed 1,000 mg/kg/day (or 20,000 ppm in the diet), 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 an untreated or sham treated group or a vehicle-control group if a vehicle is used in administering the test substance.

(B) If a vehicle is used in administering the test substance, the 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.

(D) If a test substance is administered in the diet and causes reduced dietary intake or utilization, the use of a pair-fed control group may be considered necessary.

(iii) **Route of administration.** (A) The test substance is usually administered by the oral route (diet, drinking water, or gavage).

(B) If administered by gavage or dermal application, the dosage administered to each animal prior to mating and during gestation and lactation should be based on the individual animal body weight and adjusted weekly at a minimum.

(C) 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 (g)(18) and (g)(19) of this guideline. Care should be taken to minimize stress on the maternal animals and their litters during gestation and lactation.

(D) All animals should be dosed by the same method during the appropriate experimental period.

(iv) **Dosing schedule.** (A) The animals should be dosed with the test substance on a 7–days–a–week basis.

(B) Daily dosing of the parental (P) males and females should begin when they are 5 to 9 weeks old. Daily dosing of the F1 males and females

should begin at weaning. For both sexes (P and F1), dosing should be continued for at least 10 weeks before the mating period.

(C) Daily dosing of the P and F1 males and females should continue until termination.

(3) **Mating procedure—(i) Parental.** (A) For each mating, each female should be placed with a single randomly selected male from the same dose level (1:1 mating) until evidence of copulation is observed or either 3 estrous periods or 2 weeks has elapsed. Animals should be separated as soon as possible after evidence of copulation is observed. If mating has not occurred after 2 weeks or 3 estrous periods, the animals should be separated without further opportunity for mating. Mating pairs should be clearly identified in the data.

(B) Vaginal smears should be collected daily and examined for all females during mating, until evidence of copulation is observed.

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

(ii) **F1 mating.** For mating the F1 offspring, at least one male and one female should be randomly selected from each litter for mating with another pup of the same dose level but different litter, to produce the F2 generation.

(iii) **Second mating.** In certain instances, such as poor reproductive performance in the controls, or in the event of treatment-related alterations in litter size, the adults may be remated to produce an F1b or F2b litter. If production of a second litter is deemed necessary in either generation, the dams should be remated approximately 1–2 weeks following weaning of the last F1a or F2a litter.

(iv) **Special housing.** After evidence of copulation, animals that are presumed to be pregnant should be caged separately in delivery or maternity cages. Pregnant animals should be provided with nesting materials when parturition is near.

(v) **Standardization of litter sizes.** (A) Animals should be allowed to litter normally and rear their offspring to weaning. Standardization of litter sizes is optional.

(B) If standardization is performed, the following procedure should be used. On day 4 after birth, the size of each litter may be adjusted by eliminating extra pups by random selection to yield, as nearly as possible, four males and four females per litter or five males and five females per litter. Selective elimination of pups, i.e. based upon body weight, is not appropriate. Whenever the number of male or female pups prevents having four (or five) of each sex per litter, partial adjustment (for example, five

males and three females, or four males and six females) is acceptable. Adjustments are not appropriate for litters of eight pups or less.

(4) **Observation of animals**—(i) **Parental.** (A) Throughout the test period, each animal should be observed at least once daily, considering the peak period of anticipated effects after dosing. Mortality, moribundity, pertinent behavioral changes, signs of difficult or prolonged parturition, and all signs of overt toxicity should be recorded at this cageside examination. In addition, thorough physical examinations should be conducted weekly on each animal.

(B) Parental animals (P and F1) should be weighed on the first day of dosing and weekly thereafter. Parental females (P and F1) should be weighed at a minimum on approximately gestation days 0, 7, 14, and 21, and during lactation on the same days as the weighing of litters.

(C) During the premating and gestation periods, food consumption should be measured weekly at a minimum. Water consumption should be measured weekly at a minimum if the test substance is administered in the water.

(D) Estrous cycle length and pattern should be evaluated by vaginal smears for all P and F1 females during a minimum of 3 weeks prior to mating and throughout cohabitation; care should be taken to prevent the induction of pseudopregnancy.

(E) For all P and F1 males at termination, sperm from one testis and one epididymis should be collected for enumeration of homogenization-resistant spermatids and cauda epididymal sperm reserves, respectively. In addition, sperm from the cauda epididymis (or vas deferens) should be collected for evaluation of sperm motility and sperm morphology.

(*1*) The total number of homogenization-resistant testicular sperm and cauda epididymal sperm should be enumerated (see paragraphs (g)(3) and (g)(13) of this guideline). Cauda sperm reserves can be derived from the concentration and volume of sperm in the suspension used to complete the qualitative evaluations, and the number of sperm recovered by subsequent mincing and/or homogenizing of the remaining cauda tissue. Enumeration in only control and high-dose P and F1 males may be performed unless treatment-related effects are observed; in that case, the lower dose groups should also be evaluated.

(*2*) An evaluation of epididymal (or vas deferens) sperm motility should be performed. Sperm should be recovered while minimizing damage (refer to paragraph (g)(13) of this guideline), and the percentage of progressively motile sperm should be determined either subjectively or objectively. For objective evaluations, an acceptable counting chamber of sufficient depth can be used to effectively combine the assessment of motility with sperm count and sperm morphology. When computer-assisted

motion analysis is performed (refer to paragraph (g)(13) of this guideline), the derivation of progressive motility relies on user-defined thresholds for average path velocity and straightness or linear index. If samples are videotaped, or images otherwise recorded, at the time of necropsy, subsequent analysis of only control and high-dose P and F1 males may be performed unless treatment-related effects are observed; in that case, the lower dose groups should also be evaluated. In the absence of a video or digital image, all samples in all treatment groups should be analyzed at necropsy.

(*3*) A morphological evaluation of an epididymal (or vas deferens) sperm sample should be performed. Sperm (at least 200 per sample) should be examined as fixed, wet preparations (refer to paragraphs (g)(7) and (g)(13) of this guideline) and classified as either normal (both head and midpiece/tail appear normal) or abnormal. Examples of morphologic sperm abnormalities would include fusion, isolated heads, and misshapen heads and/or tails. Evaluation of only control and high-dose P and F1 males may be performed unless treatment-related effects are observed; in that case, the lower dose groups should also be evaluated.

(ii) **Offspring.** (A) Each litter should be examined as soon as possible after delivery (lactation day 0) to establish the number and sex of pups, stillbirths, live births, and the presence of gross anomalies. Pups found dead on day 0 should be examined for possible defects and cause of death.

(B) Live pups should be counted, sexed, and weighed individually at birth, or soon thereafter, at least on days 4, 7, 14, and 21 of lactation, at the time of vaginal patency or balanopreputial separation, and at termination.

(C) The age of vaginal opening and preputial separation should be determined for F1 weanlings selected for mating. If there is a treatment-related effect in F1 sex ratio or sexual maturation, anogenital distance should be measured on day 0 for all F2 pups.

(5) **Termination schedule.** (i) All P and F1 adult males and females should be terminated when they are no longer needed for assessment of reproductive effects.

(ii) F1 offspring not selected for mating and all F2 offspring should be terminated at comparable ages after weaning.

(6) **Gross necropsy.** (i) At the time of termination or death during the study, all parental animals (P and F1) and when litter size permits at least three pups per sex per litter from the unselected F1 weanlings and the F2 weanlings should be examined macroscopically for any structural abnormalities or pathological changes. Special attention should be paid to the organs of the reproductive system.

(ii) Dead pups or pups that are terminated in a moribund condition should be examined for possible defects and/or cause of death.

(iii) At the time of necropsy, a vaginal smear should be examined to determine the stage of the estrous cycle. The uteri of all cohabited females should be examined, in a manner which does not compromise histopathological evaluation, for the presence and number of implantation sites.

(7) **Organ weights.** (i) At the time of termination, the following organs of all P and F1 parental animals should be weighed:

(A) Uterus (with oviducts and cervix), ovaries.

(B) Testes, epididymides (total weights for both and cauda weight for either one or both), seminal vesicles (with coagulating glands and their fluids), and prostate.

(C) Brain, pituitary, liver, kidneys, adrenal glands, spleen, and known target organs.

(ii) For F1 and F2 weanlings that are examined macroscopically, the following organs should be weighed for one randomly selected pup per sex per litter.

(A) Brain.

(B) Spleen and thymus.

(8) **Tissue preservation.** The following organs and tissues, or representative samples thereof, should be fixed and stored in a suitable medium for histopathological examination.

(i) For the parental (P and F1) animals:

(A) Vagina, uterus with oviducts, cervix, and ovaries.

(B) One testis (preserved in Bouins fixative or comparable preservative), one epididymis, seminal vesicles, prostate, and coagulating gland.

(C) Pituitary and adrenal glands.

(D) Target organs, when previously identified, from all P and F1 animals selected for mating.

(E) Grossly abnormal tissue.

(ii) For F1 and F2 weanlings selected for macroscopic examination: Grossly abnormal tissue and target organs, when known.

(9) **Histopathology**—(i) **Parental animals.** Full histopathology of the organs listed in paragraph (e)(8)(i) of this guideline should be performed

for ten randomly chosen high dose and control P and F1 animals per sex, for those animals that were selected for mating. Organs demonstrating treatment-related changes should also be examined for the remainder of the high-dose and control animals and for all parental animals in the low- and mid-dose groups. Additionally, reproductive organs of the low- and mid-dose animals suspected of reduced fertility, e.g., those that failed to mate, conceive, sire, or deliver healthy offspring, or for which estrous cyclicity or sperm number, motility, or morphology were affected, should be subjected to histopathological evaluation. Besides gross lesions such as atrophy or tumors, testicular histopathological examination should be conducted in order to to 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 (refer to paragraph (g)(11) of this guideline). Examination of the intact epididymis should include the caput, corpus, and cauda, which can be accomplished by evaluation of a longitudinal section, and should be conducted in order to identify such lesions as sperm granulomas, leukocytic infiltration (inflammation), aberrant cell types within the lumen, or the absence of clear cells in the cauda epididymal epithelium. The postlactational ovary should contain primordial and growing follicles as well as the large corpora lutea of lactation. Histopathological examination should detect qualitative depletion of the primordial follicle population. A quantitative evaluation of primordial follicles should be conducted for F1 females; the number of animals, ovarian section selection, and section sample size should be statistically appropriate for the evaluation procedure used.

Examination should include enumeration of the number of primordial follicles, which can be combined with small growing follicles (see paragraphs (g)(1) and (g)(2) of this guideline), for comparison of treated and control ovaries.

(ii) **Weanlings.** For F1 and F2 weanlings, histopathological examination of treatment-related abnormalities noted at macroscopic examination should be considered, if such evaluation were deemed appropriate and would contribute to the interpretation of the study data.

(f) **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 animals displaying each type of change.

(2) **Evaluation of study results.** (i) An evaluation of test results, including the statistical analysis, should be provided. This should include 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 abnormalities.

(ii) When appropriate, historical control data should be used to enhance interpretation of study results. Historical data, 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.

(iii) 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.

(iv) 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) Toxic response data by sex and dose, including indices of mating, fertility, gestation, birth, viability, and lactation; offspring sex ratio; precoital interval, including the number of days until mating and the number of estrous periods until mating; and duration of gestation calculated from day 0 of pregnancy. The report should provide the numbers used in calculating all indices.

(iii) Day (week) of death during the study or whether animals survived to termination; date (age) of litter termination.

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

(v) Developmental milestone data (mean age of vaginal opening and preputial separation, and mean anogenital distance, when measured).

(vi) An analysis of P and F1 females cycle pattern and mean estrous cycle length.

(vii) Day (week) of observation of each abnormal sign and its subsequent course.

(viii) Body weight and body weight change data by sex for P, F1, and F2 animals.

(ix) Food (and water, if applicable) consumption, food efficiency (body weight gain per gram of food consumed), and test material consumption for P and F1 animals, except for the period of cohabitation.

(x) Total cauda epididymal sperm number, homogenization-resistant testis spermatid number, number and percent of progressively motile sperm, number and percent of morphologically normal sperm, and number and percent of sperm with each identified anomaly.

(xi) Stage of the estrous cycle at the time of termination for P and F1 parental females.

(xii) Necropsy findings.

(xiii) Implantation data and postimplantation loss calculations for P and F1 parental females.

(xiv) Absolute and adjusted organ weight data.

(xv) Detailed description of all histopathological findings.

(xvi) Adequate statistical treatment of results.

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

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

(1) Bolon, B. et al. Differential follicle counts as a screen for chemically induced ovarian toxicity in mice: results from continuous breeding bioassays. *Fundamental and Applied Toxicology* 39:1-10 (1997).

(2) Bucci, T.J. et al. The effect of sampling procedure on differential ovarian follicle counts. *Reproductive Toxicology* 11(5):689-696 (1997).

(3) Gray, L.E. *et al.* A dose-response analysis of methoxychlor-induced alterations of reproductive development and function in the rat. *Fundamental and Applied Toxicology* 12:92–108 (1989).

(4) Heindel, J.J. and R.E. Chapin, (eds.). Part B. Female Reproductive Systems, *Methods in Toxicology*, Academic, Orlando, FL (1993).

(5) Heindel, J.J. *et al.* Histological assessment of ovarian follicle number in mice as a screen of ovarian toxicity. In: *Growth Factors and the Ovary*, A.N. Hirshfield (ed.), Plenum, NY, pp. 421–426 (1989).

(6) Korenbrot, C.C. *et al.* Preputial separation as an external sign of pubertal development in the male rat. *Biology of Reproduction* 17:298–303 (1977).

(7) Linder, R.E. *et al.* Endpoints of spermatoxicity in the rat after short duration exposures to fourteen reproductive toxicants. *Reproductive Toxicology* 6:491–505 (1992).

(8) Manson, J.M. and Y.J. Kang. Test methods for assessing female reproductive and developmental toxicology. In: *Principles and Methods of Toxicology,* A.W. Hayes (ed.), Raven, New York (1989).

(9) Organization for Economic Cooperation and Development, No. 416: Two Generation Reproduction Toxicity Study, Guidelines for Testing of Chemicals. [C(83)44 (Final)] (1983).

(10) Pederson, T. and H. Peters. Proposal for classification of oocytes and follicles in the mouse ovary. *Journal of Reproduction and Fertility* 17:555–557 (1988).

(11) Russell, L.D. *et al. Histological and Histopathological Evaluation of the Testis*, Cache River, Clearwater, FL (1990).

(12) Sadleir, R.M.F.S. Cycles and seasons, In: *Reproduction in Mammals*: I. Germ Cells and Fertilization, C.R. Auston and R.V. Short (eds.), Cambridge, NY (1979).

(13) Seed, J., R.E. Chapin, E.D. Clegg, L.A. Dostal, R.H. Foote, M.E. Hurtt, G.R. Klinefelter, S.L. Makris, S.D. Perreault, S. Schrader, D. Seyler, R. Sprando, K.A. Treinen, D.N.R. Veeramachaneni, and L.D. Wise. Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. *Reproductive Toxicology* 10(3):237–244 (1996).

(14) Smith, B.J. *et al.* Comparison of random and serial sections in assessment of ovarian toxicity. *Reproductive Toxicology* 5:379–383 (1991).

(15) Thomas, J.A. Toxic responses of the reproductive system. In: *Casarett and Doull's Toxicology*, M.O. Amdur, J. Doull, and C.D. Klaassen (eds.), Pergamon, NY (1991).

(16) U.S. Environmental Protection Agency. OPP Guideline 83–4: Reproductive and Fertility Effects. Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals. Office of Pesticides and Toxic Substances, Washington, DC, EPA–540/9–82–025 (1982).

(17) U.S. Environmental Protection Agency. Subpart E—Specific Organ/Tissue Toxicity, 40 CFR 798.4700: Reproduction and Fertility Effects.

(18) U.S. Environmental Protection Agency. Health Effects Test Guidelines, OPPTS 870.3250, 90-Day Dermal Toxicity, July 1998.

(19) U.S. Environmental Protection Agency. Health Effects Test Guidelines, OPPTS 870.3465, 90-Day Inhalation Toxicity, July 1998.

(20) U.S. Environmental Protection Agency. Reproductive Toxicity Risk Assessment Guidelines. **Federal Register** 61 FR 56274–56322 (1996)

(21) Working, P.K. and M. Hurtt. Computerized videomicrographic analysis of rat sperm motility. *Journal of Andrology* 8:330–337 (1987).

(22) Zenick, H. et al. Assessment of male reproductive toxicity: a risk assessment approach. In: *Principles and Methods of Toxicology*, A.W. Hayes (ed.), Raven, NY (1994).