Designation: E 1483 – 92 (Reapproved 2004)

Standard Test Method for Assessing Developmental Toxicity in Rats and Rabbits[1]

This standard is issued under the fixed designation E 1483; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method is designed to assess the potential of a pesticide or chemical to present a hazard to the unborn arising from exposure of the maternal animal during pregnancy.

1.2 This test method assumes that the user is knowledgeable in animal toxicology and related pertinent areas, and it relies heavily on the judgment of the evaluator.

1.3 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For specific hazard statements, see Section 6.

2. Referenced Documents

2.1 *ASTM Standards:* [2]

E 609 Terminology Relating to Pesticides

E 943 Terminology Relating to Biological Effects and Environmental Fate

2.2 *Federal Standards:*

Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Subchapter E, Pesticide Programs: Part 160, Good Laboratory Practice Standards[3]

Title 21, Code of Federal Regulations (CFR), Food and Drug Administration, Part 58, Good Laboratory Practice for Nonclinical Studies[3]

Title 40, Code of Federal Regulation (CFR), Toxic Substance Control Act, Part 792, Good Laboratory Practice Standards[3]

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this test method, see Terminology E 609 and E 943.

3.1.2 *developmental toxicity*—the property of a test substance that causes in utero death, structural or functional abnormalities (teratogenic), or growth retardation during the period of fetal development. To assess that property, the test substance is administered to the maternal animal during the period of major organogenesis.

4. Summary of Test Method

4.1 Rats and rabbits have been used extensively as animal models to determine whether a test substance is potentially hazardous to the developing fetus. The use of two species is desirable because of differences in maternal and fetal metabolism, placental barriers, and chemical absorption.

4.2 The test substance is administered in graded doses to groups of pregnant animals from the time of embryonic implantation through the period during which major organ systems are formed. At least 20 pregnant rats and 12 pregnant rabbits are required for each of the three test groups and the control group.

4.3 A pilot study is conducted to establish the three dose levels for the full study.

4.4 On Day 20 of gestation for rats and Day 29 for rabbits, the pregnant females are sacrificed, the uteri are removed, and the contents are examined for embryonic or fetal deaths and live fetuses. Collected fetuses are weighed, measured, tagged, and sacrificed in preparation for complete examination.

4.5 If a limit test at an exposure of least 1000 mg/kg body weight produces no observable toxicity, a full study using three dose levels might not be necessary.

5. Significance and Use

5.1 In the assessment and evaluation of the toxic characteristics of a chemical, determination of the potential developmental toxicity is significant.

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[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and Alternative Control Agents and is the direct responsibility of Subcommittee E35.26 on Safety to Man.

Current edition approved Oct. 1, 2004. Published October 2004. Originally approved in 1992. Last previous edition approved in 2000 as E 1483 – 92 (2000).

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from U.S. Government Printing Office, Superintendent of Documents, Washington, DC 20402.

5.2 This test method is intended to permit the determination of a no-observed-effect level and the potential hazard to the unborn that may arise from exposure of the pregnant organism during gestation.

6. Hazards

6.1 Contact with all test substances and solutions should be minimized with appropriate protective clothing, gloves, eye protection, etc. The use of fume hoods and increased ventilation in test rooms is necessary when handling volatile substances. Information on acute mammalian toxicity and special handling procedures for all chemicals and products to be used should be known before this test method is employed.

6.2 The disposal of excess test substance, solutions, diets, excreta, and treated animals should be performed with consideration for health and environmental safety, and in accordance with all federal, state, and local regulations.

7. Facilities

7.1 No precise physical requirements concerning animals are set forth. However, the animal facility shall meet the established standard that may be required by law or regulations. It is desirable that the animal facilities meet the guidelines suggested by the Institute of Laboratory Resources or facilities that have been approved by such organizations as the American Association for Accreditation of Laboratory Animal Care (AAALAC).

8. Procedure for Rat Studies

8.1 *Animals*—Virgin, sexually mature rats should be used to produce pregnancies. The Sprague-Dawley (COBS/CD) rat is an example of a strain frequently used. The rats shall undergo an acclimation period of no less than 14 days before study startup. In no case should rats be less than 9 weeks or older than 12 weeks of age when mating commences.

8.2 *Environment*—House test and control rats individually in all metal cages designed to hold laboratory rats. These cages should provide for proper water and food consumption. Maintain all rats in a temperature-, humidity-, and light-controlled room. The conditions should be 18 to 26°C (64.4 to 78.8°F) for temperature, 40 to 70 % for humidity, and a 12-h light, 12-h dark lighting cycle.

8.3 *Mating Procedure*—For mating purposes, house rats together, with one male and one female per cage. The morning following the initiation of cohabitation, check the cage pads for the presence of sperm plugs. The finding of a plug(s) under a cage shall be construed as a positive mating. That day is then designated as that female's Day "0" of gestation. (A second mating option is housing two females per male, with vaginal smear observations required.) Each positively mated female will then be randomly assigned to a dose group and housed individually as above. Cage cards shall convey the dam number, day of mating, dose group, mating group, and day of sacrifice. Change the cage pads daily during the mating procedure. As positively mated females are removed from the males, replace them with virgin females. Continue this procedure until all dose groups have been filled with an appropriate number of animals. Mating groups should contain no more animals than make it possible to maintain a manageable number of fetal exams on any given sacrifice day. When all dose groups have been filled, sacrifice all males, excess non-mated females, and excess mated females by CO_2 inhalation or other humane means, and discard them in the appropriate manner.

8.4 *Pilot Study*—To establish dose levels for a full developmental toxicity study, a pilot study must be performed for each test substance.

8.4.1 At least 36 female rats, mated as described, should be distributed randomly among five treatment groups and one negative control. In the case of oral administration, doses for the five treatment groups should be chosen as arithmetic fractions of the oral LD_{50} for the test substance. The negative control group will receive diluent vehicle at a volume approximating that of the highest dose group. Alternately, compound may be administered by the dermal or inhalation route if either is the only expected mode of human exposure. Follow the dosing (8.7), observation (8.8), and necropsy (8.9) procedures.

8.5 *Limit Test*—If a test at an oral exposure of at least 1000 mg/kg body weight, using the procedures described for this study, produces no observable developmental toxicity, a full study using three dose levels might not be necessary.

8.6 *Dose Groups*—Base the three dose levels on the results of the pilot study. The highest dose group should be sufficient to produce some observable maternal toxicity while causing no more than 10 % maternal deaths. The lowest dose group must produce no observable maternal or fetal toxicity and, ideally, should be no less than one-tenth of the high dose. A mid level should also be selected, and it should be approximately one-half of the high dose. A negative control group must be run concurrently with the three treatment groups. The control group will usually receive diluent vehicle only at a volume approximating that of the high dose group.

8.6.1 Randomly place at least 25 positively mated females in each of the four groups.

8.7 *Dosing*—Dose the mated females with the appropriate materials, starting on their sixth day of gestation (Day 6). Continue the dosing once per day, at the same time each day, through Day 15 of gestation. Unless indicated otherwise in an approved study plan, dosing will be accomplished by oral gavage. Calculate daily doses based on the individual female's body weight obtained on Day 6. The daily doses will remain constant throughout the dosing period.

8.8 *Observations*—Observe all rats grossly at least once per day. Record signs of toxicity as they are observed, including the time of onset, degree, and duration. Record the individual body weight for all mated females on Day 0, 6, 10, 13, 16, and 20 of gestation. Monitor the food consumption for each test animal by weighing and refilling the feed containers on those days that the mated females are weighed. Both "off" and "on" weights should be recorded. Record anorexic rats and decide whether the animals should remain in the study. Any female that is found dead, moribund, or aborts a litter shall be euthanized, if living, and subjected to a complete gross necropsy.

8.9 *Necropsy*—On Day 20 of gestation, sacrifice each female, in turn, by CO_2 inhalation or other humane means.

Remove the gravid uterus and weigh, after which record the counts and location of corpora lutea, implantations, resorptions, and dead and live fetuses. Assign the fetuses an arbitrary number starting at the upper right uterine horn and continuing to the upper left horn. After opening the uterus, each viable fetus will then be removed, examined externally, sexed, and weighed. At this time, examine the female (dam) grossly for any structural abnormalities or pathological changes that may have influenced the pregnancy. Record all necropsy data.

8.10 *Fetal Examinations*[4]—Immediately following the gross necropsy of the female, place each fetus in a numbered compartment to maintain identity on the original uterine position. In turn, decapitate the even-numbered fetuses using a large scissor. Place the head in a numbered plastic vial, covered with Bouin's fluid and capped. The vials, with one fetal head per vial, will then be placed collectively in a heavy plastic bag labeled with the study number, dam number, dose group, and collection date. Fix the heads at least one week before examination. Secure the remainder of each even-numbered fetus and all odd-numbered fetuses to an examination board with pins or rubber bands for dissection. Record all soft tissue observations. Following the dissection and removal of internal organs, each fetus shall be skinned, tagged for identification, and placed in a small plastic bottle containing denatured alcohol. Following a fixation period of no less than 10 days, stain the skeletons and then examine them for malformations, completeness, and degree of ossification. Record all observations. The examination of fetal heads following fixation should consist of a series of transverse sections (five or six) viewed under a dissecting scope to detect anomalies of the brain, nasal cavities, eyes, and palate. Record all information from the examination of the fetal heads.

9. Procedure for Rabbit Study

9.1 *Animals*—Obtain rabbits from a reputable breeding laboratory. The New Zealand White rabbit is an example of a strain used frequently. All rabbits shall be 22 to 26 weeks of age and free of hepatic coccidiosis, enteric coccidiosis, treponema cuniculi, and pasteurella multocida. For pilot studies, the rabbits shall be nonpregnant, but they may be exbreeders. For full developmental studies, the rabbits shall be nulliparous and time-mated at the breeder's facility. Mated animals will be delivered to the facility in such manner to ensure that no more than 12 females (does) will be sacrificed on any given day and that the sacrifice days will not fall on holidays or non-work days. Identify all of the rabbits uniquely upon arrival.

9.2 *Environment*—House the rabbits individually in appropriate wire cages. Maintain all rabbits in a temperature-, humidity-, and light-controlled room. The conditions should be 16 to 21°C (61 to 69.8°F) for temperature, 50 ± 5 % for humidity, and a 12-h light, 12-h dark lighting cycle.

9.3 *Pilot Study*—Perform a pilot study for each test substance to establish the dose levels for a full developmental toxicity study.

9.3.1 Distribute 28 nonpregnant female rabbits randomly among six treatment groups and one negative control group. Each group shall contain four females. Select the doses for the six treatment groups as arithmetic fractions of the LD_{50} for the test substance. Continue with the dosing (9.6), observation (9.7), and necropsy (9.8) procedures.

9.4 *Limit Test*—If a test at an exposure of at least 1000 mg/kg body weight, using the procedures described in this test method, produces no observable developmental toxicity, a full study using three dose levels might not be necessary.

9.5 *Dose Groups*—Base the three dose levels for a full study on the results of the pilot study.

9.5.1 The highest dose shall be sufficient to produce some observable maternal toxicity while causing no more than 10 % maternal deaths. The lowest dose shall produce no observable maternal or fetal toxicity and, ideally, shall be no less than one-tenth of the high dose. The control group will usually receive diluent vehicle only at a volume approximating that of the high dose group.

9.5.2 Place at least 18 mated females randomly in each of the four dose groups.

9.6 *Dosing*—For pilot studies, dose the non-mated female rabbits with the appropriate materials once per day for 13 consecutive days. For full studies, dose the mated rabbits on their sixth day of gestation (Day 6). Dosing will continue once per day, at the same time each day, through Day 18 of gestation. Unless indicated otherwise in an approved study plan, dosing will be accomplished by oral gavage. Calculate the daily doses based on the individual female's body weight obtained on the first day of dosing, and this weight will be used throughout the dosing period.

9.7 *Observations*—Observe all of the rabbits grossly at least once per day. Record all signs of toxicity, including the time of onset, degree, and duration. Record the individual body weights for all females on Days 6, 10, 13, 16, 19, 24, and 29 of gestation (or the equivalent days for non-mated rabbits). Monitor the food consumption for each test animal by weighing the food container every other day from Day 5 through Day 29 (or the equivalent). When the feeders require refilling, record both the "off" and "on" weights. Record the anorexic rabbits and decide whether those animals will remain in the test. Any female that is found dead, moribund, or aborts a litter shall be euthanized, if living, and subjected to a complete gross necropsy.

9.8 *Necropsy*—On Day 29 of gestation (or the equivalent), sacrifice each female, in turn, by CO_2 inhalation or other humane means. In the case of nonpregnant pilot study female rabbits, gross necropsy will consist of an examination for macroscopic pathological changes. Record all findings. For pregnant rabbits, the gravid uterus will be removed and weighed, after which record the counts and location of corpora lutea, implantations, resorptions, and dead and live fetuses. Assign the fetuses an arbitrary number starting at the does' upper right uterine horn and continuing to the upper left horn. After opening the uterus, remove each viable fetus, examine externally, and weigh. At this time, examine the female (doe)

[4] R. E. Staples, "Detection of Visceral Alterations in Mammalian Fetuses," *Teratology*, Vol 9, 1974, pp. A37–A38 (as adapted by the National Center for Toxicological Research, 1984).

grossly for any structural abnormalities or pathological changes that may have influenced the pregnancy. Record all necropsy data.

9.9 *Fetal Examinations*—Immediately following the necropsy of the female, place each fetus in a numbered compartment of a divided plastic tray to maintain its identity regarding the original uterine position. In turn, decapitate the even-numbered fetuses using a laboratory guillotine. Place the head in a numbered, divided plastic box and cover with Bouin's solution. When the box has accommodated the heads of one or two litters, close the box tightly and tape it shut. Place a label on the lid over the heads of a litter, recording the study number, doe number, dose group, and collection date. Fix the heads for at least two weeks before examination. Secure the remainder of each even-numbered fetus and all odd-numbered fetuses to an examination board with rubber bands for dissection. Record all soft tissue observations. Following the dissection and removal of internal organs, each fetus shall be skinned, tagged for identification, and placed in a large plastic bottle containing denatured alcohol. Stain the skeletons following a fixation period of no less than two weeks. After staining, examine the fetal skeletons for malformations, completeness, and degree of ossification. Record all observations. The examination of fetal heads following fixation should consist of a series of transverse sections (five or six) viewed under a dissecting scope to detect anomalies of the brain, nasal cavities, eyes, and palate. Record all data from the examination of fetal heads.

10. Quality Assurance

10.1 To ensure the quality and reliability of data developed using this test method, appropriate laboratory practices should be followed (see CFR Titles 21, Part 58, and 40, Parts 160 and 792).

11. Data Handling

11.1 Consider the following parameters, by test groups, when the study groups are analyzed and comparisons are made among test groups:

11.1.1 Fertility index (= No. of pregnant/No. of positively mated × 100),

11.1.2 Gestation index (= No. of with viable litter/No. of pregnant × 100),

11.1.3 Index of live fetuses (= No. of live fetuses/total fetuses × 100),

11.1.4 Resorption index (= No. of resorptions/No. of implantations × 100),

11.1.5 Index of variation (= No. of fetuses with variations/total No. of fetuses × 100),

11.1.6 Index of malformation (= No. of fetuses with malformations/total No. of fetuses × 100),

11.1.7 Maternal body weights,

11.1.8 Maternal food consumption,

11.1.9 Fetal body weights,

11.1.10 Implantations per pregnancy,

11.1.11 Fetuses per pregnancy,

11.1.12 Dead fetuses per pregnancy,

11.1.13 Resorptions per pregnancy,

11.1.14 Variant fetuses per pregnancy,

11.1.15 Malformed fetuses per pregnancy, and

11.1.16 Fetal sex ratio (male/female).

12. Report

12.1 Report the following information:

12.1.1 Name of investigator(s), laboratory, laboratory address, location of raw data, and date of initiation and termination of the test.

12.1.2 Name of species and strain of animals tested, including scientific name, source, and age of the animals at the beginning of the test.

12.1.3 Detailed description of the test substance, including its chemical name, Chemical Abstracts Services (CAS) name, synonyms, structure, formulations, purity, source, batch or lot number, physical/chemical properties, and name of solvent or carrier (purity, brand, and batch number), if used.

12.1.4 Description of the test facilities and housing conditions, including test cages, temperature, humidity, and photoperiod.

12.1.5 Name and source of feeds, including a description and analysis of the diet.

12.1.6 Concentration of the test substance in solvent or carrier; calculated doses for each test group.

12.1.7 Number of animals per dose group, body weights, food consumption, signs of toxicity (numbers affected by dose groups), abnormal behavior, necropsy findings, and a detailed description of all histopathological findings.

12.1.8 Pregnancy and litter data, fetal data (live/dead, sex, soft tissue and skeletal defects, and resorptions).

12.1.9 Anything unusual concerning, the test, any deviations from protocol, and other relevant information.

12.1.10 Statistical methods used.

13. Precision and Bias

13.1 A precision and bias statement cannot be made at this time for this test method.

14. Keywords

14.1 developmental toxicity; fetuses; gestation; malformations; oral; pesticide; rabbits; rats; resorption; toxicity