Designation: E 1291 – 99 (Reapproved 2003)

Standard Test Method for Conducting a Saturated Vapor Inhalation Study with Rats[1]

This standard is issued under the fixed designation E 1291; 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 estimates the relative hazard of handling a liquid chemical, pesticide, or mixture, where exposure to vapors from spilled liquids may result.

1.2 The results of this test method may also be used to evaluate and compare the relative hazard between two liquid chemicals.

1.3 This test method measures hazard rather than quantitative toxicity because the amount inhaled is governed by vapor pressure. It is recognized that the vapor air mixture in this test is not completely saturated, although for brevity it is known as saturated vapor.

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

1.5 *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.* See Section 7 for additional hazard information.

2. Referenced Documents

2.1 *ASTM Standards:*

E 609 Definitions of Terms Relating to Pesticides[2]

E 943 Terminology Relating to Biological Effects and Environmental Fate[2]

2.2 *Federal Standards:*[3]

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

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

Title 40, Code of Federal Regulations (CFR), Toxic Substance Control Act, Part 792, Good Laboratory Practice Standards

3. Terminology

3.1 *vapors*—the gaseous forms of compounds or mixtures which are normally in the liquid or solid state.

3.2 *vapor pressure*—the pressure characteristic at any given temperature of a vapor in equilibrium with its liquid or solid form.

4. Summary of Test Method

4.1 Two groups of six male rats each are placed in 9-L glass desiccators that serve as inhalation chambers.

4.2 Compressed air at 1 L/min is bubbled through the test material in a gas-washing bottle and then passed through the inhalation chamber. The exposure time is 8 h. One bottle holds the test liquid at room temperature and the other bottle is held at 100° C in a hot oil bath.

4.3 A third group of six rats is placed in another desiccator and has only compressed air passing through it. This is the control group.

4.4 Upon completion of the exposure, the gas-washing bottles are weighed again and the nominal concentration is expressed as a ratio of the test material expelled to the total volume of air delivered in each test chamber.

4.5 On Day 14 postexposure, the surviving rats are sacrificed, examined for gross abnormalities, and a complete necropsy done.

5. Significance and Use

5.1 This test method determines the potential inhalation hazard of a liquid material where exposure consists of inhaling vapors.[4]

5.2 The results of this test method may be used to compare and evaluate the relative vapor hazard between two liquid materials.

5.3 This test method is also applicable to materials whose melting point is slightly above room temperature.

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

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[2] *Annual Book of ASTM Standards*, Vol 11.05.

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

[4] Carpenter, C. P., Smyth Jr., H. F., and Pozzani, U. C., "The Assay of Acute Toxicity, and the Grading and Interpretation of Results on 96 Chemical Compounds," *Journal of Industrial Hygiene and Toxicology*, Vol 31, 1949, pp. 343–346.

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5.4 Results of this test method can provide information for conducting acute and subchronic inhalation studies.

6. Apparatus

6.1 *Inhalation Chambers*:

6.1.1 Use three large glass desiccators (9-L), each to hold one group of animals. Place a 2-hole rubber stopper in the top of each desiccator. Insert a 6.3-mm (¼-in.) diameter glass tube (inlet) through the stopper to extend down to approximately 25.4 mm (1 in.) from the bottom of the chamber and to protrude 50.8 mm (2 in.) above the stopper. Insert another glass tube (outlet) to extend approximately 6.3 mm (¼ in.) below and 25.4 mm (1 in.) above the stopper.

6.1.2 House the animals in two flat, circular-wire cages designed to fit within the desiccators.

6.2 *Dispersion Apparatus*:

6.2.1 Use two 125-mL gas-washing bottles with fritted disk or cylindrical ends. One bottle will contain the test material at room temperature and the other bottle will contain test material held at 100°C in a hot oil bath on a hot plate with a variable transformer as a control.

6.2.2 If the test material has a melting point slightly higher than room temperature, the bottle and test material shall be heated in an oil bath placed on a hot plate. If the test material is highly volatile, the bottle and test material are placed in a water bath at room temperature to prevent evaporative cooling.

6.3 *Airflow*:

6.3.1 Use compressed air for this test method. Pass the air through an air filter, a single stage pressure regulator, and then a canister of desiccant. The air line shall branch into three separate lines. Connect two lines each to rotometers that are attached to the inlet tubes of the gas-washing bottles and the inhalation chambers. The air lines should be kept as short as possible to avoid condensation of the vapors. The third line is connected directly to a rotometer and the control chamber.

6.4 Place all the equipment in a fume hood designed to handle hazardous materials.

6.5 *Calibration*:

6.5.1 Before the actual exposure, calibrate airflow to provide equal flow to each chamber. Turn on the air source and adjust the pressure regulator to 68.947 kPa (10 psi). Measure individually the airflow from the rotometers with a wet test meter. Adjust each rotometer to a flow of 1 L/min and record.

6.5.2 Determine the setting of the heating system to hold 100°C. Place a 1-L beaker approximately half full of mineral oil on the hot plate. Place a thermometer in the oil and turn on the hotplate and variable transformer. Adjust the transformer so that the desired temperature remains constant for at least 1 h, and record the setting.

7. Hazards

7.1 Contact with all test substances, solutions, and mixed diets, 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 other mammalian toxicity and special handling procedures should be known before this test method is used.

7.2 Disposal of excess test substances, solutions, mixed diets, excreta, and treated animals should be done with consideration for health and environmental safety, and in agreement with all federal, state, and local regulations.

7.3 Cleaning and rinsing of glassware, feeders, and other equipment with volatile solvents should be done only in well-ventilated areas.

7.4 Periodic medical examinations should be considered for all personnel caring for animals or handling test substances.

8. Test Animals

8.1 This test method is intended for use with young albino male rats weighing 90 to 100 g. A non in-bred rat such as the Sprague-Dawley strain is generally preferred. Rodents other than rats may be used with appropriate modifications and justifications.

8.2 All animals for a given test must come from one source and strain and be of approximately the same age to minimize variability. Test animals may be obtained from commercial sources or reared in laboratory colonies, but they must not have been used in a previous test. Animals should be healthy and disease free and those that are deformed, injured, emaciated, or phenotypically different from normal animals, must not be used as test subjects. The population of animals from which the test subjects (treated and control) are selected shall be considered unsuitable for testing if mortality exceeds 5 % during the acclimation period. At the beginning of the study the weight variation of the rats used should not exceed ±20 % of the mean weight.

8.3 A total of 18 male rats comprise 2 test groups and 1 control group of 6 per group.

9. Pretest Conditions

9.1 Examine each test animal on arrival for overt signs of disease, and condition to the environment for a minimum of 7 days. Select animals that have not been used for other tests.

9.2 Maintain animals during pretest and test periods in agreement with accepted laboratory practices for the care and handling of test animals.

9.3 Identify each animal with ear tag or other suitable means.

9.4 During acclimation, observe the animals for adverse health effects. Eliminate any animal(s) showing signs of spontaneous disease before the start of the study. Use only animals judged to be healthy.

10. Procedure

10.1 Weigh test and control animals immediately before placement in the chambers. Compare body weights of test animals to the body weights for the control animals to ascertain statistical insignificance among groups.

10.2 Place rats in the inhalation chambers and replace the chamber covers.

10.3 Fill 2 gas-washing bottles to a level approximately 6 cm above the fritted end of inlet tube with the test material. Weigh each bottle to the nearest 0.01 g and record.

10.4 Connect the gas-washing bottles into the system.

10.5 With the rotometers closed, turn on the air source and adjust the pressure regulator to 68.947 kPa (10 psi). Open the rotometers to their proper setting and start a timer.

10.6 The exposure shall last 8 h; during this time observe all animals periodically for toxic signs.

10.7 Upon completion of the exposure, weigh each gas-washing bottle to determine the nominal concentration in each test chamber. Nominal concentration is expressed as a ratio of test material expelled to the total volume of air delivered (mg/L).

10.8 Observation of the animals should be made throughout the test period and at least once each day following the exposure, with appropriate actions taken to minimize loss of animals to the study (for example, necropsy or refrigeration of animals found dead, and isolation or sacrifice of weak or moribund animals). Any animal that becomes moribund or succumbs shall be euthanized and examined for any gross abnormalities; preserve all tissues and organs in 10 % formalin for microscopic examination.

10.9 Signs of toxicity (per group) should be recorded as they are observed, including time of onset, degree, and duration. These signs include, but are not limited to, changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, central nervous system, and unusual behavioral patterns.

10.10 All animals must be weighed before initial exposure on Day 1, and on the 3rd, 7th, and 14th day postexposure.

10.11 On the 14th day postexposure, sacrifice all animals by accepted humane methods and subject to a gross necropsy. This shall include examination of the external surface of the body, all orifices, and the cranial, thoracic, and abdominal cavities, and their contents. Conduct a gross examination for abnormalities; weigh brain, lungs, liver, spleen, heart, kidneys, and testes and record. Before being weighed, organs should be carefully dissected and trimmed to remove fat and other tissue in a uniform manner. Organs should be weighed as soon as possible to avoid drying.

10.12 The following organs and tissues, or representative samples should be preserved in a 10 % formalin for future histopathological examination: A sample of all tissues containing gross lesions; brain (including sections of medulla/pons, cerebellar cortex and cerebral cortex); pituitary; thyroid parathyroid; thymus; lungs and trachea; heart; bone marrow (either femur, sternum or rib) at the costochondral junction; salivary glands; liver; spleen; kidney; adrenals; pancreas; testes; uterus; aorta; esophagus; stomach; duodenum; jejunum; ileum; caecum; colon; rectum; urinary bladder; representative lymph node and peripheral nerve.

10.13 The following tissues need be preserved only if indicated by signs of toxicity or target organ involvement: trachea; sternum with bone marrow; mammary gland; thigh musculature; eyes; femur (including articular surface); spinal cord at three levels (cervical, midthoracic, and lumbar) and exorbital lachrymal glands.

10.14 *Histopathology*:

10.14.1 Conduct full histopathological examinations on organs and tissues of all animals in the control and high dosage groups and all animals that died or were killed during the study.

10.14.2 Perform histopathological examinations on all gross lesions and on lungs, liver, and kidneys of all animals.

10.14.3 Conduct further histopathology on organs in other dosage groups that show lesions in the high dosage group or for which clinical observations indicate such a need.

10.15 Compare statistically test group data (animal weights, organ-to-body weight, organ-to-brain weight ratios, (or appropriate alternative means of correlation), hematology, and clinical chemistry and uranalysis), for any given period with the control group data for the same period. Generally any acceptable statistical method may be used.

11. Quality Assurance

11.1 In order to assure the quality and reliability of data developed using this test method, good laboratory practices should be followed (see 2.2).

12. Interpretation of Results

12.1 Test group data (animal weights, organ-to-body weight and organ-to-brain weight ratios) will be statistically compared to the control group. Generally any test method acceptable statistically, may be used.

12.2 The statistical method should be chosen during the design of the study. Supplementary statistical tests may be performed. The need for and the nature of these tests may be determined only when the analytical results have been subjected to a preliminary examination.

13. Report

13.1 Report the following information:

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

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

13.1.3 A detailed description of the test substance including its chemical name, Chemical Abstract Services, (CAS) number, synonyms, structure, formulations, purity, source batch, lot number, physical/chemical properties.

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

13.1.5 Name and source of feed, including description and analysis of diet.

13.1.6 Individual rat body weights, organ weights, organ-to-body weight, and organ-to-brain weight ratios, mortality, pharmacotoxic signs, gross necropsy results, and microscopic examination results.

13.1.7 Total amount of test material expelled into chambers and nominal test material concentration.

14. Precision and Bias

14.1 A precision and bias statement cannot be made at this time.

15. Keywords

15.1 chemicals; inhalation; inhalation chamber; pesticides; rats; saturated vapor; vapor

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