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Designation: E 1373 – 01

Standard Test Method for Conducting a Subchronic Inhalation Toxicity Study in Rats[1]

This standard is issued under the fixed designation E 1373; 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 determines the no observed-effect level (NOEL) and toxic effects associated with continuous or repeated inhalation exposure to a chemical, pesticide, or mixture for a period of 90 days. The test is not capable of determining those effects that have a long latency period for development (for example, carcinogenicity and life shortening).

1.2 This test method which is applicable to a gas, vapor, aerosol, or particulate is conducted after initial information on toxicity is obtained by acute testing.

1.3 *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.*

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:*

OPPTS 870-3465, EPA 712-C98-204 90 day Inhalation Toxicity. Health Effects Test Guidelines, Office of Prevention, Pesticides and Toxic Substances , US Environmental Protection Agency (USEPA)[3]

Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Subchapter E, Pesticide Programs; Part 160, Good Laboratory Practice Standards[4]

Title 21, Code of Federal Regulations (CFR), Food and Drug Administration, Part 58, Laboratory Practice for Nonclinical Laboratory Studies[4]

Title 40, Code of Federal Regulations (CFR), Toxic Substance Control Act, Part 792, Good Laboratory Practice Standards[4]

3. Terminology

3.1 *Definitions*—Refer to Definitions E 609 and Terminology E 943.

3.1.1 *aerodynamic diameter*—The diameter of the unit density sphere that has the same terminal settling velocity due to gravity as the particle under consideration, whatever its size, shape and density. It is used to compare particles of different size and densities and to predict where in the respiratory tract such particles may be deposited.[5,6]

3.1.2 *concentration*—The amount of test substance administered via inhalation for a period of 90 days. Express concentration as weight of the test substance per unit volume of air (for example, milligrams per liter, parts per million).

3.1.3 *geometric mean diameter or median diameter*—The calculated aerodynamic diameter that divides the particles of an aerosol in half, based on the weight of the particles. Fifty percent of the particles by weight will be larger than the median diameter and 50 % of the particles will be smaller than the median diameter. The median diameter describes the particle size distribution of any aerosol based on the weight and size of the particles.

3.1.4 *geometric standard deviation*—The measure of dispersion for a log-normal distribution, the ratio of the 84.13 percentile to the 50 percentile and 50 percentile to the 15.8 percentile.

3.1.5 *inhalable diameter*—The aerodynamic diameter of a particle that is considered to be inhalable for the organism. It is used to refer to particles that are capable of being inhaled and deposited anywhere within the respiratory tract from the trachea to the alveoli. For man, inhalable diameter is considered as 15 µm or less (see CFR Title 40, Part 798).

3.1.6 *cumulative toxicity*—The adverse effects caused when the inactivation or excretion, or both, of a substance is slower than the rate at which the substance is being administered.

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

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[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 Environmental Protection Agency Ariel Rios Building 1200 Pennsylvania Avenue, N.W. Washington, DC 20460 (202) 260-2090

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

[5] Hinds, William C., *Aerosol Technology, Properties, Behavior, and Measurement of Airborne Particles*, John Wiley & Sons, 1982.

[6] Richard, D., *Handbook on Aerosols*, U.S. Energy Research and Development Administration, 1976.

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Since there is more of the substance entering the body than is being removed, a build up results, causing repeated dosage of the substance to produce a more marked response than after the first dose.

3.1.7 *no-observed-effect-level (NOEL)*—The maximum concentration used in a study that produces no observed effects.

3.1.8 *subchronic inhalation toxicity*—The adverse effects occurring as a result of the repeated daily exposure of experimental animals to a chemical by inhalation for a part (approximately 10 %) of a life span.

4. Summary of Test Method (see Literature [7])

4.1 Three groups of 20 rats (10 female, 10 males) each are used for at least three dose levels. Doses should be spaced appropriately to produce test groups with a range of toxic effects and mortality rates.

4.2 A concurrent control group of 20 rats should be untreated or sham treated.

4.3 An additional group of 20 rats may be treated with the high concentration level for 90 days and then observed for a post-treatment period normally not less than 28 days.

4.4 Rats are exposed to the test substance, ideally for 6 h/day on a 7-day/week basis, for a period of 90 days. However, based primarily on practical considerations, exposure on a 5-day/week basis is considered to be acceptable.

4.5 Rats are placed in inhalation equipment with a dynamic air flow of 12 to 15 air changes/h. Nasal or head only exposure may be used if it is desirable to avoid exposure by the dermal or oral route.

4.6 The test substance is introduced into the chamber air supply. A suitable analytical control system should be used.

4.7 Daily observations of all individual rats for signs of toxicity and mortality are recorded.

4.8 All rats are weighed at least once every week and these weights recorded throughout the study.

4.9 Urine and blood samples are collected for analysis just prior to necropsy. After 90 days of exposure the surviving rats are weighed, sacrificed, and a complete necropsy done. Histopathological examinations are also performed on selected tissues.

5. Significance and Use

5.1 This test method determines the subchronic inhalation toxicity that is the adverse effect occurring as a result of the repeated daily exposure of experimental rats to a chemical by inhalation for part (approximately 10 %) of a life span.

5.2 This test method provides information on health hazards likely to arise from repeated exposures by the inhalation route over a limited period of time. It will provide information on target organs, the possibilities of accumulation, and can be of use in selecting dose levels for chronic studies and for establishing safety criteria for human exposure.

5.3 Hazards of inhaled substances are influenced by their inherent toxicity and by physical factors such as volatility and particle size.

6. Exposure Apparatus [8]

6.1 The inhalation chamber should sustain a dynamic air flow of 10 air changes/h and ensure adequate oxygen content of 19 % and an evenly distributed exposure atmosphere. Animals should occupy less than 5 % of the chamber volume.

6.2 A dynamic inhalation system with a suitable analytical concentration control system should be used. The rate of air flow should be adjusted to ensure that conditions throughout the chamber are essentially the same. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas.

6.3 The exhaust from the chambers must be treated appropriately to prevent the release of the test materials into the environment.

6.4 The temperature at which the test is performed should be maintained at 22°C (±2°C). Ideally, the relative humidity should be maintained between 40 to 60 %, but in certain instances (for example, tests of aerosols, use of water vehicles), this may not be practicable.

6.5 Measurements or monitoring should be made as follows:

6.5.1 The rate of air flow may be monitored continuously but should be recorded at least every 60 min,

6.5.2 The actual concentration of the test substance should be measured in the breathing zone. During the exposure period the actual concentration of the test substance should be held as constant as practicable, monitored continuously if possible or at least measured at the beginning, at an intermediate time and at the end of the exposure period, and

6.5.3 During the development of the generating system, particle size analysis should be performed to establish the stability of the aerosol particle size generation in the inhalable range. During exposure, analysis should be conducted as often as necessary (at least weekly) to determine the consistency of particle size distribution.

7. Hazards

7.1 In case of potentially explosive test substances, care should be taken to avoid generating explosive concentrations.

7.2 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. Exhaust from fume hoods and chambers must be treated appropriately to prevent the release of the test material into the environment. Information on acute mammalian toxicity and special handling procedures should be known before this test method is used.

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

7.4 Cleaning and rinsing of glassware, feeders, and other equipment with volatile solvents should be performed only in

[7] Leong, Basil K. J., *Inhalation Toxicology and Technology*, Ann Arbor Science, 1981.

[8] Silver, S. D., "Constant Gassing Chambers: Principles Influencing Design and Operation," *Journal of Laboratory Clinical Medicine*, Vol [illegible]

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