Designation: E 1674 – 99

Standard Test Method for Cardiac Sensitization Study on Dogs[1]

This standard is issued under the fixed designation E 1674; 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 covers an inhalation procedure to investigate the cardiac sensitization of volatile chlorinated hydrocarbons and other volatile solvents.

1.2 This test method is primarily a screening tool. The procedure does permit a rank order of the sensitization potential of the compounds tested, but is not recommended for establishing significant effect levels.

1.3 This test method assumes that the user is knowledgeable in mammalian toxicology, electrocardiography with animals and other pertinent areas, and relies heavily on the judgment of the investigator.

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.*

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]

3. Terminology

3.1 *Definitions*—for terms used in this test method, see Definitions E 609 and Terminology E 943.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *cardiac sensitization*—the reaction of the heart to adrenaline (epinephrine) resulting in the production of ventricular arrhythmias.

4. Summary of Test Method [3]

4.1 Six healthy male dogs are employed for each test material. After the initial acclimation period, the dogs are trained to maintain a standing position while supported in a cloth sling and wearing an inhalation mask over their nose and mouth.

4.2 Since dogs can vary markedly in their cardiac response to intravenous injection of adrenaline (epinephrine), each dog is evaluated to select an appropriate dose. The selected dose is used in all subsequent work.

4.3 All six dogs are tested on the same day, one at a time. Each animal is placed in the sling, fitted with the inhalation mask and standard electrocardiogram (ECG) leads connected to an electrocardiograph.

4.4 The test proceeds as follows:

4.4.1 At zero time start the ECG recording.

4.4.2 At 2 min, administer the selected dose of adrenaline into the cephalic vein.

4.4.3 At 7 min, start the inhalation of vapor of the test material. Monitor the concentration of test material administered.

4.4.4 At 12 min, administer a challenge injection of adrenaline.

4.4.5 At 17 min, stop the vapor inhalation and the ECG recording.

4.4.6 Return the dogs to their kennels. They should be tested on a Monday, Wednesday, and Friday regimen.

4.5 The dogs are exposed to a 2.5 % concentration of the test material the first time; then exposed to 5 % on the next test day. If necessary, the dogs are exposed successively to 10 %, 15 %, and 20 % as needed. As soon as ventricular tachycardia is evidenced, the testing is stopped. If no appearance of ventricular arrhythmia occurs in the ECG recording at the highest concentration and there are no other deleterious events, then no cardiac sensitization has occurred.

5. Significance and Use

5.1 This test method is designed to provide information as to any dose level of test material that gives rise to clear signs of compound-induced cardiac sensitization.

5.2 Cardiac sensitization has occurred in humans during periods of stress while being exposed to high concentrations of some unsubstituted and halogenated hydrocarbons.[3]

5.3 The dog is employed for this study because the conclusions from this test method can be applied to man with much

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[1] This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.26 on Safety to Man.

Current edition approved October 10, 1999. Published November 1999. Originally published as E 1674 - 95. Last previous edition E 1674 - 95.

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

[3] Reinhardt, C. F., Azar, A., Maxfield, M. E., Smith, P. E. Jr., and Mullin, L. S., "Cardiac Arrhythmias and Aerosol Sniffing," *Archives of Environmental Health*, Vol. 22, No. 2, February 1971, pp. 265–279.

more certainty than those from other animals.[4]

6. Facility

6.1 No precise physical requirements concerning facilities are set forth. However, the animal facility shall meet the established standard(s) 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).

6.2 *Environment*—House animals in kennels to hold laboratory animals. Maintain all animals 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.

7. Test Animal

7.1 Use six healthy, male beagle dogs, 13 to 26 months old, weighing 7 to 14 kg (15.4 to 30.9 lb) for each test material to be evaluated. Keep the dogs in a laboratory kennel for 14 days to acclimate them.

7.2 Feed the dogs daily with a standard dog chow and provide tap water at all times, except when they are placed in experimental sessions. The dogs that are used for experiments will be fed on their return to their kennels.

8. Procedure

8.1 Train each dog individually to accept restraint in a canvas holding sling over a period of four weeks. Training will take place on three days (Monday, Wednesday, and Friday) of each week. During Week 1, restrain each dog in the sling and an inhalation mask for approximately 30 min each day. During Week 2, restrain for approximately 45 min, and during Weeks 3 and 4, restrain for 1 h per day.

8.1.1 Any dog that has failed to accept the restraint procedure to the extent that useful data cannot be obtained will not be used.

8.1.2 Each dog will receive additional training on at least one day per week in which it is not undergoing exposure until the end of the study.

8.2 After the four weeks of training, evaluate each dog for its cardiac response to intravenous injection of adrenaline since it can vary markedly among dogs.

8.2.1 Place the animal in the sling and fit it with standard ECG leads connected to an electrocardiograph. Hold the electrodes in place on the dog by a rubber strap extending around the body and position over shaved areas as follows:

8.2.1.1 Two active electrodes, one on each side of the chest just behind the foreleg.

8.2.1.2 One indifferent electrode, over the sternum.

8.2.1.3 Run the ECG at a speed (at least 10 mm/s) sufficient to allow identification of ectopic beats following adrenaline challenge.

8.2.2 Administer the initial dose of adrenaline intravenously into the cephalic vein on the foreleg. Make the injection by an automatic infusion pump set to deliver the total dosage of 0.008 mg adrenaline/kg of body weight in a volume of 0.1 ml of normal saline per kg body weight; deliver at the rate of 0.1 mL/sec.

8.2.2.1 If the response to the first adrenaline challenge is too small (as defined by no ectopic beats) or too large (as defined by an excessive number of ectopic beats; more than approximately 10), end the experimental session and allow the animal to rest at least 20 min.

8.2.2.2 Repeat the procedure with an adrenaline dose either of 0.004 mg adrenaline/kg body weight or 0.012 mg adrenaline/kg body weight, based upon the prior response.

8.2.3 When a suitable dose of adrenaline that produces one to five ectopic beats per min has been selected for each dog, that dose will be used for all subsequent work.

8.3 Test all six dogs individually on the same day; test on a Monday, Wednesday, and Friday regimen. Place the dog in the sling and attach the inhalation mask through which either vapor of the test material or fresh air can be passed. Attach the standard ECG leads to the dog and to the electrocardiograph. Run the ECG at a speed sufficient to allow identification of ectopic beats.

8.3.1 At zero min of the test, start the ECG recording. Run the ECG recording at a speed of at least 10 mm/s.

8.3.2 At 2 min into the test, administer adrenaline (0.1 mL saline solution/kg body weight containing an appropriate adrenaline dilution) at approximately 0.1 mL/s into a cephalic vein.

8.3.3 At 7 min, start the inhalation of vapor of the test material by metering the gas into the dog's air supply. Monitor continuously the concentration of test substance in the air breathed by the animal during exposure by a suitable analytical procedure.

8.3.4 At 12 min, administer a second dose of adrenaline (0.1 mL/kg of an appropriate dilution).

8.3.5 At 17 min, stop the vapor inhalation and ECG recording.

8.3.6 If positive response is observed or if the dog is in distress before 17 min into the test, then stop the vapor inhalation and ECG recording at that time. Note that fact on the ECG chart.

8.3.7 After the test is completed, return the animal to its kennel.

8.4 Expose the dogs to a 2.5 % concentration of the test material the first test day. Then, if no response, expose to a 5 % concentration on the next test day. If necessary, expose successively to 10 %, 15 %, and 20 % as needed. As soon as ventricular arrhythmia is evidenced, stop the testing.

8.5 The dogs may be used in further tests of additional test materials.

9. Interpretation of Results

9.1 The appearance of ventricular arrhythmia (a series or sum of unifocal ectopic beats) is not always definitive evidence of compound induced cardiac sensitization. The criterion for a positive effect in this study should be the appearance of five or

[4] Meek, W. J., Hathaway, H. R., and Orth, O. S., "The Effects of Ether, Chloroform and Cyclopropane on Cardiac Automaticity," *Journal of Pharmacology and Experimental Therapy*, Vol. 61, 1937, pp. 240–252.

more multifocal ventricular ectopic beats or ventricular fibrillation. However, make the interpretation of the results for each dog based on its response to the initial dose for adrenaline.

9.2 If no appearance of ventricular arrhythmia occurs above those expected from epinephrine administration alone in the ECG recording at the highest concentration and there are no deleterious events, then no cardiac sensitization has occurred.

10. Report

10.1 Report the following information:

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

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

10.1.3 Detailed description of the test substance including its chemical name, Chemical Abstracts Services (CAS) number, synonyms, structure, formulations purity, source batch, lot number, physical/chemical properties.

10.1.4 Description of test facilities and housing conditions, including test cages, vapor generation system, monitoring equipment and adrenaline administration.

10.1.5 Name and source of feed including description and analysis of diet.

10.1.6 The concentration of test substance administered and the predicted dose for each test group. Describe the system of test substance administration and the procedure employed for monitoring the concentration.

10.1.7 The results of each exposure to vapor and the effective dosage of adrenaline for each animal.

10.1.8 Anything unusual about the test, any deviations from the protocol and any other relevant information.

11. Quality Assurance[5]

11.1 Utilize good laboratory practices to ensure the quality and reliability of data developed using this test method.

12. Precision and Bias

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

13. Keywords

13.1 adrenaline; cardiac sensitization; chlorinated hydrocarbons; dogs; inhalation; pesticides; solvent; toxicity

[5] Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Subchapter E, Pesticide Programs: Part 160, Good Laboratory Practice Standards, Available from U.S. Government Printing Office, Superintendent of Documents, Washington, DC 20402.