



Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals¹

This standard is issued under the fixed designation E981; 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 laboratory test method provides a rapid means of determining sensory irritant potential of airborne chemicals or mixtures. It may also be used to estimate threshold limit values (TLV) for man. However, it cannot be used to evaluate the relative obnoxiousness of odors.

1.2 This test method is intended as a supplement to, not a replacement for, chronic inhalation studies used to establish allowable human tolerance levels.

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.* Specific hazard information is given in Section 6.

2. Summary of Test Method

2.1 This test method quantitatively measures irritancy as indicated by the reflex inhibition of respiration in mice exposed to sensory irritants.

2.2 Four mice are simultaneously exposed to the airborne chemical. Usually a sufficient number of groups of animals are exposed to a geometric series of concentrations so that a concentration-response curve can be constructed. For simple preliminary comparisons, however, a single group of four animals at one concentration will suffice.

2.3 The mice are placed in a body plethysmograph attached to an exposure chamber so that only the head is exposed to the test material. The plethysmographs are connected to pressure transducers, which sense changes created by inspiration and expiration. The amplified signals are transmitted to a polygraph recorder.

2.4 The concentration of airborne irritant that produces a 50 % decrease in respiratory rate (RD50) is determined from the concentration-response curve constructed from the various data points obtained with a series of concentrations.

¹ This test method is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.02 on Terrestrial Assessment and Toxicology.

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3. Significance and Use

3.1 This test method was developed to meet the following criteria:

3.1.1 It provides positive recognition of sensory irritants of widely varying potencies.

3.1.2 It is sufficiently simple to permit the testing of large numbers of materials.

3.1.3 This test method is capable of generating concentration-response curves for purposes of compound comparison.

3.1.4 This test method has good reproducibility.

3.2 This test method can be used for a variety of divergent purposes, including the assessment of comparative irritancy of compounds or formulations and setting interim exposure levels for the workplace (**1, 2**).²

3.3 It has been shown that for a wide variety of chemicals and mixtures, a perfect rank order correlation exists between the decreases in respiratory rate in mice and subjective reports of sensory irritation in man (**1, 3, 4, 5**).

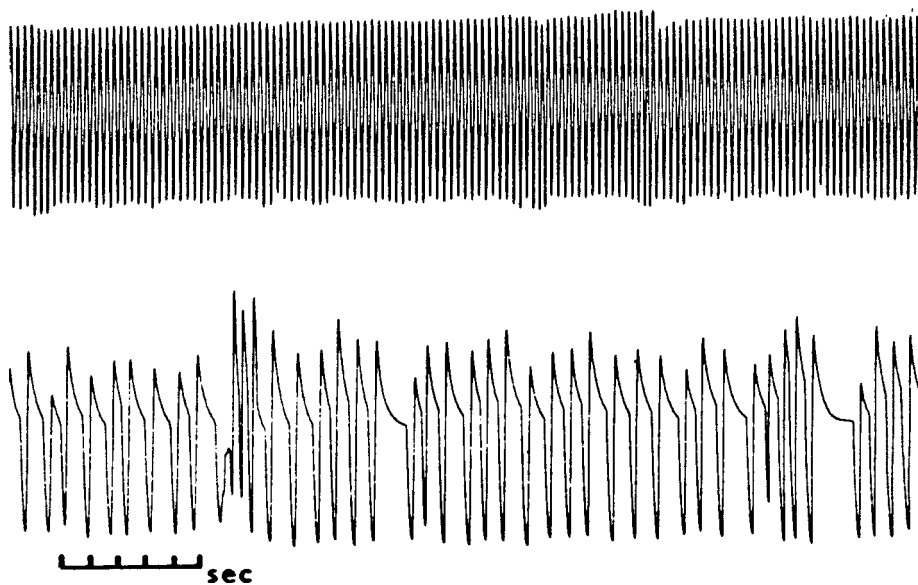
3.4 A quantitative estimate of the sensory irritancy of a wide variety of materials can be obtained from concentration-response curves developed using this method (**1, 3, 4, 6, 7, 8, 9**).

3.5 Although this test method is intended to measure sensory irritation of the nasal mucosa, the cornea is innervated by the same nerve. This animal model will, therefore, allow an estimate of the irritant potential of cosmetic ingredients or other household products to the eye, assuming that they can be aerosolized (**10**).

3.6 This test method is recommended for setting interim guidelines for exposure of humans to chemicals in the workplace, to assess acute sensory irritation resulting from inadvertent spills of household products, and to assess the comparative irritancy of formulations or materials intended for a variety of uses (see **Appendix X2**).

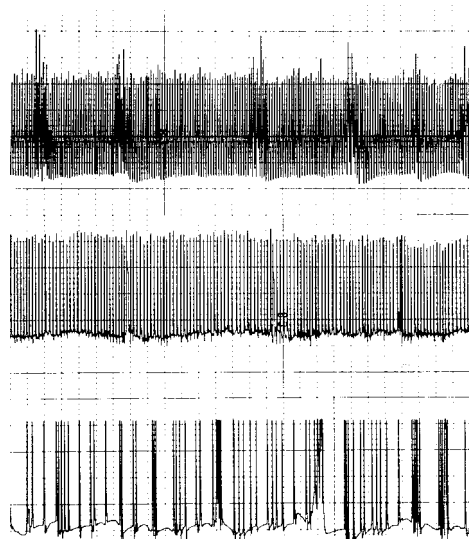
3.7 This test method will detect irritating effects at concentrations far below those at which pathological changes are observed (**9**).

² The boldface numbers in parentheses refer to the list of references at the end of this standard.



NOTE 1—Taken from Ref. (3).

FIG. 1 Typical Tracing of Normal Mouse Respiration (Top), and of a “Moderate” Sensory Irritant Response (Bottom)



NOTE 1—Taken from Ref. (8).

FIG. 2 Typical Tracing of Normal Mouse Respiration (Top), a Moderate Pulmonary Irritant Response (Center), and an Extreme Pulmonary Irritant Response (Bottom)

NOTE 1—A good overview of the toxicological evaluation of irritant compounds is given in Ref (8).

4. Apparatus

4.1 The apparatus required to perform this test is listed below. The basic components for testing any type of material are the same. A list of suitable apparatus and suppliers is found in Appendix X1.

4.2 *Plethysmograph Tubes.*

4.3 *Exposure Chamber*, constructed entirely of glass, with a volume of 2.3 L.

4.4 *S.T.103/60 Ground Glass Joint*, that allows access to the inside of the exposure chamber.

4.5 *Perforated Rubber Dental Dam*, reinforced with electrical tape.

4.6 *Rubber Stoppers.*

4.7 “T” *Tube*, with a tube 6 cm long and the “T” 12 cm long.

4.8 *Vacuum Pump.*

4.9 *Flowmeter.*

4.10 *Absolute Filter.*

4.11 *Sodium Carbonate-Activated Charcoal Filter.*

4.12 *Pressure Transducer.*

4.13 *Polygraph Recorders.*

4.14 *Frequency-to-Voltage Converter*, operating in the averaging mode instead of the pulse mode. See Appendix X1.7.

4.15 *Voltage Addition and Division Equipment*, to obtain the signal average for four mice.

4.16 *Signal Averages.*

4.17 *Oscillograph.*

4.18 *Aerosol Generator.*

4.19 *Timer.*

4.20 *Control Valve.*

5. Reagents

5.1 Technical reagents may be used in all tests where solvents other than water are required.

5.2 Solutions containing 1 to 3 % of the test material are used for comparative studies.

6. Hazards

6.1 Not all compounds that cause a decrease in respiratory rate are sensory irritants. To be characterized as a sensory irritant, a compound must produce a net decrease in respiratory rate as a result of the characteristic pause during expiration as

shown in Fig. 1. This pause differentiates sensory irritants from pulmonary irritants, general anesthetics, and asphyxiants, which also reduce respiratory rate, but as a result of a pause between breaths as shown in Fig. 2.

6.2 It is possible for one component to alter the effect of another in a mixture, depending on their respective concentrations (11). Additive and antagonistic responses are possible. For this reason the effects of each compound in a formulation should be assessed before any test is made for interactions.

6.3 Although the test procedure has been found to show a high correlation for sensory irritants with established TLV values for man, it may well predict values that are too high for compounds of low reactivity that are metabolically activated, and also for pulmonary irritants (10).

7. Test Animals

7.1 Mice are the subjects to be used for this test. It is imperative that they meet the specifications outlined here. Although any mouse of the proper size could be used, marked differences have been observed between different strains and sexes (2).

7.1.1 Male Swiss-Webster mice shall be used as the test subjects.

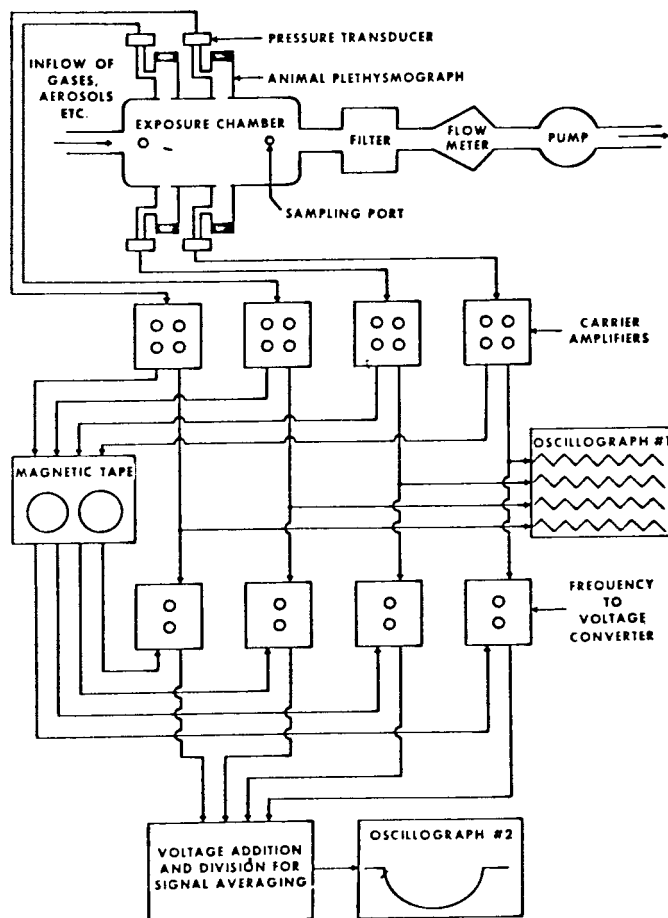
7.1.2 Only animals weighing between 22 and 28 g may be used. Smaller mice might be able to crawl into the exposure chamber, while larger ones may not be able to breathe normally in the apparatus.

7.1.3 The same system can be used with guinea pigs or rats with an airflow of 2 L/min when using head dome (9).

8. Preparation of Apparatus

8.1 Exposure Chamber:

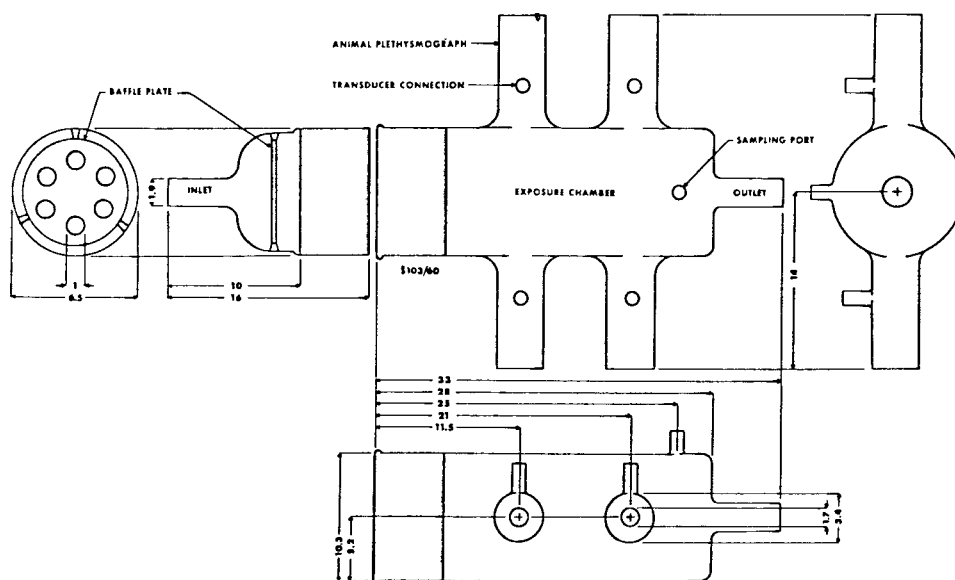
8.1.1 The heads of each of four mice extend into the exposure chamber, and the bodies are contained in plethysmograph tubes. Perforated rubber dental dam reinforced with



NOTE 1—Taken from Ref. (19).

FIG. 4 Diagram of Test Apparatus

electrical tape provides tight but comfortable seals around the



NOTE 1—Dimensions are in centimetres.

NOTE 2—Taken from Ref. (19).

FIG. 3 Glass Exposure Chamber with Attached Body Plethysmographs