



Designation: F750 – 20

# Standard Practice for Evaluating Acute Systemic Toxicity of Material Extracts by Systemic Injection in the Mouse<sup>1</sup>

This standard is issued under the fixed designation F750; 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 ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

## 1. Scope

1.1 This practice covers a nonspecific, acute toxicity test used for detecting leachables from materials used in medical devices.

1.2 The liquids injected into the mouse are those obtained by Practice F619 where the extraction vehicles are saline, vegetable oil, or other liquids simulating human body fluids.

1.3 Two procedures are outlined: Method A for intravenous injection and Method B for intraperitoneal injection.

1.4 This practice is one of several developed for the assessment of the biocompatibility of materials. Practice F748 may provide guidance for the selection of appropriate methods for testing materials for a specific application.

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.6 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

## 2. Referenced Documents

2.1 *ASTM Standards:*<sup>2</sup>

F619 Practice for Extraction of Medical Plastics

<sup>1</sup> This practice is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.16 on Biocompatibility Test Methods.

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<sup>2</sup> 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.

F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices

## 3. Summary of Practice

3.1 The extract liquid is prepared in accordance with Practice F619. The extraction vehicles are saline and vegetable oil, or other extraction vehicles, as described in Practice F619. The extract liquid is injected into mice, and the animals are observed at regular intervals for 72 h for reactions, survival, etc.

## 4. Significance and Use

4.1 This practice is intended to help assess the biocompatibility of materials used in medical devices. It is an acute toxicological test designed to detect the presence of injurious leachable substances.

4.2 This practice may not be appropriate for all types of implant applications. The user is cautioned to consider the appropriateness of the method in view of the materials being tested, their potential applications, and the recommendations contained in Practice F748.

4.3 The only limitation applicable is the extract preparation. Refer to Sections 4.3 and 4.4 of Practice F619 for a description of this limitation.

## 5. Apparatus

5.1 *Mice*—The mice shall be albino-type, healthy, and not previously used, and shall weigh between 17 and 23 g. Animal care shall be in accordance with the “Guide for the Care and Use of Laboratory Animals.”<sup>3</sup> Age, sex, and weight shall be recorded and reported. All the mice for each extraction vehicle shall be from the same source. For each extraction vehicle, a minimum of ten mice are used in the test. If the results of this first test group are inconclusive, then 20 more mice will be needed to complete the test of one extraction vehicle for one plastic.

5.1.1 During the test the mice shall be fed normally with commercially available feed and tap water.

<sup>3</sup> National Research Council, *Guide for the Care and Use of Laboratory Animals*, National Academy Press, Washington, DC, 2011.

5.2 *Cages*—There shall be one cage for the five mice exposed to one extract liquid. Each mouse in a cage shall be uniquely identified, and this identification shall be recorded. Male and female mice shall be housed separately, and their cages positioned in a manner which prevents the accidental transfer of feces or bedding from cage to cage.

5.3 *Syringe*—Sterile syringes, not greater than 3 mL in volume, with a precision of  $\pm 0.10$  mL shall be used.

5.3.1 *Method A*—Sterile needles of 25 to 27½ gauge shall be used.

5.3.2 *Method B*—Sterile needles of 21 to 26 gauge shall be used.

## 6. Sampling

6.1 Sample in accordance with Practice F619.

## 7. Sample and Test Specimen

7.1 *General*—The sample is the plastic or other material exposed to the extraction procedure. As a result of the extraction in Practice F619, for each extraction vehicle there shall be available: (1) a sample extract liquid, and (2) a blank extract liquid. These extract liquids shall be injected into the test animals within 24 h of the end of the extraction procedure. Record the storage conditions if the liquid extract is not used immediately after preparation.

7.1.1 There are usually two extract liquids (a blank and a sample) prepared from an extraction vehicle. Samples based on other extraction vehicles may be available, as described in Practice F619, or as required by the standard for the medical device.

### 7.2 *Method A, Intravenous:*

7.2.1 The extract liquid is usually prepared from a saline extraction vehicle. The dose of the extract liquid is 50 mL/kg of body weight for each mouse, injected at a steady rate of not more than 0.1 mL/s.

7.2.2 Aqueous extract liquids shall be nominally isotonic before injection. For example, sodium chloride may be added to distilled water extracts.

7.3 *Method B, Intraperitoneal*—The extract liquid is prepared from a vegetable oil extraction vehicle. The dose of the extract liquid is 50 mL/kg of body weight for each mouse.

## 8. Procedure

### 8.1 *Method A, Intravenous:*

8.1.1 Use saline and similar extraction vehicles designated for intravenous injection.

8.1.2 Agitate each extract liquid vigorously prior to withdrawal of each injection dose to ensure even distribution of the extracted matter. If particulates are present, allow the particulates to settle. If they do not settle, consider injecting them by the intraperitoneal route and provide justification.

NOTE 1—pH may be measured and recorded as particulate formation may result in a pH change.

8.1.3 For each extraction vehicle, use ten mice, five for the sample extract liquid and five for the blank extract liquid.

Weigh all mice, and record their weights. Use a system of marking to identify each individual mouse within each group of five.

8.1.4 Inject the predetermined amount (see 7.2.1) of the sample extract liquid into the tail vein of each of the five mice. Inject the blank extract liquid in the same way into five other mice. The use of warm water or a heat lamp may help dilate the tail veins for ease of injection.

8.1.5 Observe all animals immediately after injection, again 4 h after injection, and then at 24, 48, and 72 h, respectively, after injection for clinical signs. Cage-side observations should include, but not be limited to, changes in body condition, skin/hair coat, gait/posture, eyes, respiratory, gastrointestinal, and consciousness/mentation using the descriptors provided in Table 1. Record the observations. Measure and record the body weights of all animals at 24, 48, and 72 h post-injection.

### 8.2 *Method B, Intraperitoneal:*

8.2.1 Method B shall be used with vegetable oil and similar extraction vehicles designated for intraperitoneal injection.

8.2.2 Agitate each extract liquid vigorously prior to withdrawal of each injection dose to ensure even distribution of extracted matter. If the extract liquid contains particulates, record and report observations.

8.2.3 For each extraction vehicle use ten mice, five for the sample extract liquid and five for the blank extract liquid. Weigh all mice, and record their weight. Use a system of marking to identify each individual mouse within each group of five.

8.2.4 Inject the predetermined amount (see 7.3) of the sample extract liquid intraperitoneally into each of the five mice. Inject the blank extract liquid in the same way into five other mice.

8.2.5 Observe all animals immediately after injection, again 4 h after injection, and then not earlier than 24, 48, and 72 h, respectively, after injection for clinical signs. Cage-side observations should include, but not be limited to, changes in body condition, skin/hair coat, gait/posture, eyes, respiratory, gastrointestinal, and consciousness/mentation using the descriptors provided in Table 1. Record the observations. Measure and record the body weights of all animals at 24, 48, and 72 h post-injection.

## 9. Interpretation of Results

### 9.1 *Initial Test:*

9.1.1 *Acceptable Result*—If during the 72-h observation period none of the animals treated with the sample extract liquid shows a substantially greater biological reaction than the animals treated with the blank extract liquid, the sample meets the requirements of the test.

NOTE 2—Any abnormal behavior in the blank control group should be justified.

9.1.2 *Adverse Response*—Among five animals, if  $\geq 2$  mice die, or have marked symptoms of toxicity or abnormal behavior, such as convulsions or prostration occurs as defined in Table 1, the sample does not meet the requirements of the test. If a body weight loss of  $>10\%$  occurs in  $\geq 3$  mice at the end of the study, the sample does not meet the requirements of the test. Any moribund animals should be removed and

**TABLE 1 Mouse Clinical Signs and Grading Scheme<sup>A,B,C</sup>**

Organ System	Normal	Slight	Moderate	Marked
Body Condition	Well fleshed and muscled and bones are palpable but not prominent	Slender body conformation but well fleshed and good muscling	Thin body conformation, bone prominence, minimal adipose tissue; weight loss of 10–15 %	Muscle wasting, emaciation and cachexia, no fat deposits; weight loss of 20 %
Hair Coat	Well-groomed, normal pelage	Slightly unkempt; mild alopecia; transient piloerection	Unkempt and ungroomed; hair coat thinning with moderate alopecia	General loss of fur and whiskers; continuous piloerection
Gait or Posture	No lameness with normal weight bearing, and locomotion	Mild lameness or intermittent weight bearing	Hunched posture, ataxia, circling	Lateral recumbency, head tilt
Ocular	Normal anatomy. Clear lens, cornea, conjunctiva, sclera. No lesions	Mild epiphora, edema; transient squinting	Moderate conjunctivitis, corneal opacity, ptosis, clear ocular discharge; continuous squinting	Eyes sunken, purulent or hemorrhagic ocular discharge, corneal ulceration
Respiratory	Normal inspiratory and expiratory rate and pattern. No discharge from nasal or oral passages	Sneezing, mild nasal discharge	Hyperventilation; dyspnea, excessive nasal discharge	Open mouth breathing, cyanosis
Gastrointestinal	Normal eating and defecation	Transient salivation; transient diarrhea	Anorexia and/or diarrhea for 24 h, abdominal mass or swelling/enlargement of organs/tissues	Anorexia for more than 24 h; large or necrotic rectal prolapse
Integument	Normal pigment, turgor, and thickness. No lesions	Mild erythema	Ulcerative dermatitis, skin swellings, bleeding lesions; dry, flaky, oily/greasy	Excessive rubbing (face, body, extremities); self-mutilation or self-barbering; ulcerative dermatitis covering 10–20 % of skin
Consciousness & Mentation	Well groomed, active, alert, with inquisitive behavior. Normal food and water consumption	Mild hypo- or hyperactivity; decrease interest in environmental conspecifics	Highly unusual hypo- or hyperactivity; vocalization; lack of interest in environmental conspecifics	Seizures; prostration, convulsions; aggression; head tilt, moribund

<sup>A</sup> Burkholder, T., Foltz, C., Karlsson, E., Linton, C. G., and Smith, J. M., "Health Evaluation of Experimental Laboratory Mice," *Current Protocols in Mouse Biology*, Vol 2, No. 2, 2012, pp. 145–165.

<sup>B</sup> Foltz, C. J., and Ullman-Cullere, M. H., "Guidelines for Assessing the Health and Condition of Mice," *Lab Animal*, Vol 28, No. 4, 1999, pp. 28–32.

<sup>C</sup> Fentener van Vlissingen, J. M., Borrens, M., Girod, A., Lelovas, P., Morrison, F., and Torres, Y. S., "The Reporting of Clinical Signs in Laboratory Animals: FELASA Working Group Report," *Laboratory Animals*, Vol 49, No. 4, 2015, pp. 267–283.

<https://standards.iteh.ai/catalog/standards/sist/18ff3595-3250-4151-92dd-85cf525d919a/astm-f750-20>

euthanized when noticed to exhibit such behavior. Methods used for euthanasia should be in accordance with national animal welfare guidelines.

NOTE 3—Any death of animals should be justified.

### 9.2 Retest:

9.2.1 *Retest Methods*—If any animals injected with the sample show slight signs of toxicity, and not more than one animal shows moderate (for example, excessive nasal discharge, diarrhea for 24 h, bleeding lesions, etc.) or marked signs of toxicity (for example, prostration, cyanosis, convulsions, weight loss of  $\geq 20\%$ ) or dies, repeat the test using groups of ten test and ten blank extract liquid mice each.

9.2.2 *Acceptable Result*—In this repeated test, the requirements of the test are met if none of the animals injected with the sample shows a substantially greater reaction than that observed in the animals injected with the blank.

9.3 A retest (see 9.2) requires that the extraction procedure be done a second time, since the extraction fluids must be used within 24 h of the end of the extraction.

## 10. Report

10.1 Describe the sample that was extracted, including: generic name, trade name, manufacturer's code, lot number, catalog number, date of manufacture, formulation, fabrication procedures or processes, and so forth, as appropriate. Similarly, describe the extraction vehicle and the conditions of the extraction (temperature and time).

10.2 Report the number of mice used: each mouse's weight, sex, and age; and whether the mouse was exposed to the sample or blank extract.

10.3 Report whether a retest was necessary and if so, the reasons for the retest.