Designation: F750 – 87 (Reapproved 2012)

Standard Practice for Evaluating Material Extracts by Systemic Injection in the Mouse[1]

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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.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

F619 Practice for Extraction of Medical Plastics

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 Care and Use of Laboratory Animals."[3] 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.

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 are 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 ±0.10 mL shall be used.

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

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[1] 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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[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] U.S. Department of Health, Education, and Welfare, *Guide for Care and Use of Laboratory Animals*, Publication No. NIH 78-23, Bethesda, MD, 1978.

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5.3.2 *Method B*—Sterile needles of 21 to 26 gage 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 are available: (*1*) a sample extract liquid, and (*2*) a blank extract liquid. These extract liquids are to 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. If a hypotonic or hypertonic extract liquid is used, then the injection rate shall be adjusted appropriately.

7.2.2 Aqueous extract liquids shall be nominally isosmotic 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 clearly present, then the extract liquids shall be injected by the intraperitoneal method. Optionally, the pH may be measured and recorded.

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 symptoms of slight, moderate, or marked toxicity or death (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 is to 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.

TABLE 1 Response to Systemic Injection Assay

Response	Description
Normal, no symptoms	Mouse exhibits no adverse physical symptoms after injection.
Slight	Mouse exhibits slight but noticeable symptoms of hypokinesia, dyspnea, or abdominal irritation after injection.
Moderate	Mouse exhibits definite evidence of abdominal irritation, dyspnea, hypokinesia, ptosis, or diarrhea after injection. (Weight usually drops to between 15 and 17 g.)
Marked	Mouse exhibits prostration, cyanosis, tremors, or severe symptoms of abdominal irritation, diarrhea, ptosis, or dyspnea after injection. (Extreme weight loss; weight usually less than 15 g.)
Dead, expired	Mouse dies after injection.

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 symptoms of slight, moderate, or marked toxicity or death (Table 1). Record the observations. Measure and record the body weights of all animals at 24, 48, and 72 h postinjection.

9. Interpretation of Results

9.1 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.

9.2 *Reaction*—If two or more animals either show marked symptoms of toxicity or die, then the sample does not meet the requirements of the test.

9.3 *Retest*—If any animals injected with the sample show slight signs of toxicity, and not more than one animal shows marked symptoms of toxicity or dies, repeat the test using groups of ten mice each. A substantial decrease in body weight for all animals in the group, even without other symptoms of toxicity, requires a retest using groups of ten mice each. 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.

9.4 A retest (see 9.3) 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.

10.4 For each mouse used, including those used in a retest, report the clinical signs and the extract response: whether the reaction was none, slight, moderate, marked, or death. This applies to all mice, whether injected with the sample extract liquid or with the blank extract liquid.

11. Precision

11.1 *Precision*—Intralaboratory and interlaboratory reproducibility has not been systematically determined. Reproducibility may be inferred from previous round robin studies.[4,5]

12. Keywords

12.1 acute toxicity tests; biocompatibility; intravenous injection; intraperitoneal injections; mouse/mice; test animals

[4] Brewer, John H., "Toxicity Standards for Plastics," *Bulletin Parenteral Drug Assn*, Vol 19, 1965, pp. 22–28.

[5] Materials Science Toxicology Laboratories, University of Tennessee Center for the Health Sciences, Memphis, Tenn., "Determination of Levels of Chemical Purity for Biomaterials Used as Surgical Implants," Round Robin Evaluation of Primary Acute Toxicity Screening Protocols, *Quarterly Report No. 15–16*, Part II, Contract No. FDA 223-73-5231, 1978.

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