~~Designation:D4638–03 (Reapproved 2007)~~ Designation: D4638 – 11

Standard Guide for Preparation of Biological Samples for Inorganic Chemical Analysis[1]

This standard is issued under the fixed designation D4638; 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 guide describes procedures for the preparation of test samples collected from such locations as streams, rivers, ponds, lakes, estuaries, oceans, and toxicity tests and is applicable to such organisms as plankton, mollusks, fish, and plants.

1.2 The procedures are applicable to the determination of volatile, semivolatile, and nonvolatile inorganic constituents of biological materials. Analyses may be carried out or reported on either a dry or wet basis.

~~1.3~~

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

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.* For a specific hazard statement, see 9.3.3.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

3. Terminology

3.1 *Definitions*—For definitions of terms used in this guide, refer to Terminology D1129.

4. Summary of Guide

4.1 Samples are collected, where possible, with nonmetallic or TFE-fluorocarbon-coated sampling equipment to prevent contamination, stored in plastic containers, and kept either at 4°C or frozen until returned to an adequate facility for analysis.

4.2 Before analysis, samples are allowed to return to room temperature. Large foreign objects are mechanically removed from the samples based upon visual examination; smaller foreign objects are also removed mechanically, with the aid of a low-power microscope.

4.3 Wet samples of small organisms such as plankton, are mixed for preliminary homogenization, then allowed to settle, to remove most of the occluded water. Larger organisms, such as fish, should be patted dry, using paper towels.

4.4 Where less than a whole organism is to be analyzed, tissue excisions are made with nonmetallic tools such as plastic knives or TFE-fluorocarbon-coated scalpels.

4.5 Moisture determinations are made on separate samples from those analyzed for volatile or semivolatile constituents.

4.6 Analyses for volatile constituents are made using wet samples from which supernatant liquid or occluded water has been removed (see 4.3). The results may be calculated to the dry, original-sample basis, using the results of a moisture determination carried out on a separate sample.

4.7 Analyses for semivolatile constituents are made on wet samples or samples previously dried at a temperature (dependent on constituents of interest), or using a procedure, found to be adequate for the purpose, and specified in the corresponding analytical procedure.

4.8 Analyses for nonvolatile constituents are made on samples previously dried at a temperature (dependent on constituents of interest), or using a procedure found to be adequate for the purpose, and specified in the corresponding analytical procedure.

4.9 Digest the samples according to the procedures outlined in Section 9.

---

[1] This guide is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.05 on Inorganic Constituents in Water.

Current edition approved ~~Dec.~~Sept. 1, ~~2007.~~2011. Published ~~January 2008.~~September 2011. Originally approved in 1986. Last previous edition approved in ~~2003~~2007 as D4638 – 03(07). DOI: ~~10.1520/D4638-03R07.~~10.1520/D4638-11.

[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.

***A Summary of Changes section appears at the end of this standard.**

4.10 A flow diagram outlining typical procedures is shown in Fig. 1.

5. Significance and Use

5.1 The chemical analysis of biological material, collected from such locations as streams, rivers, lakes, and oceans can provide information of environmental significance. The chemical analysis of biological material used in toxicity tests may be useful to better interpret the toxicological results.

5.2 Many aquatic biological samples, either as a result of their size, or their method of collection, are inherently heterogeneous in that they may contain occluded water in varying and unpredictable amounts and may contain foreign objects or material (for example, sediment) not ordinarily intended for analysis, the inclusion of which would result in inaccurate analysis.

5.3 Standard methods for separating foreign objects, to facilitate homogenization, will minimize errors due to poor mixing and inclusion of extraneous material.

5.4 Standardized procedures for drying provide a means for reporting analytical values to a common dry weight basis, if desired. Analyses may also be carried out or reported on a wet weight basis.

6. Preliminary Treatment of Samples

6.1 Treat small heterogeneous samples, such as plankton, as follows:

6.1.1 Allow for the sample to return to room temperature.

6.1.2 Remove foreign objects, such as leaves and twigs, mechanically, using nonmetallic instruments. Use a low-power microscope to facilitate removal of smaller foreign objects such as paint chips.

6.1.3 Transfer the sample to a beaker and thoroughly mix it with a glass stirring rod or equivalent, and allow it to settle so that most or all of the occluded water can be decanted.

6.1.4 If chemical analyses are to be carried out on a wet sample, and a large amount of material is available, remove a number of small portions (at least five) from random locations in the beaker, and composite them to obtain a representative sample of a size sufficient for chemical analysis and a separate moisture determination. Using a tissue disrupter, blender, or equivalent, homogenize the sample or composite (to ensure lack of contamination, carry a standard or blank, or both, through this procedure). Remove a subsample for moisture determination and proceed to Section 7. Retain the remainder and proceed to Section 9.

6.1.5 If chemical analyses are to be carried out on a dry sample, and a large amount of material is available, remove a number of small portions (at least five) from random locations in the beaker, and composite them to obtain a representative sample of a

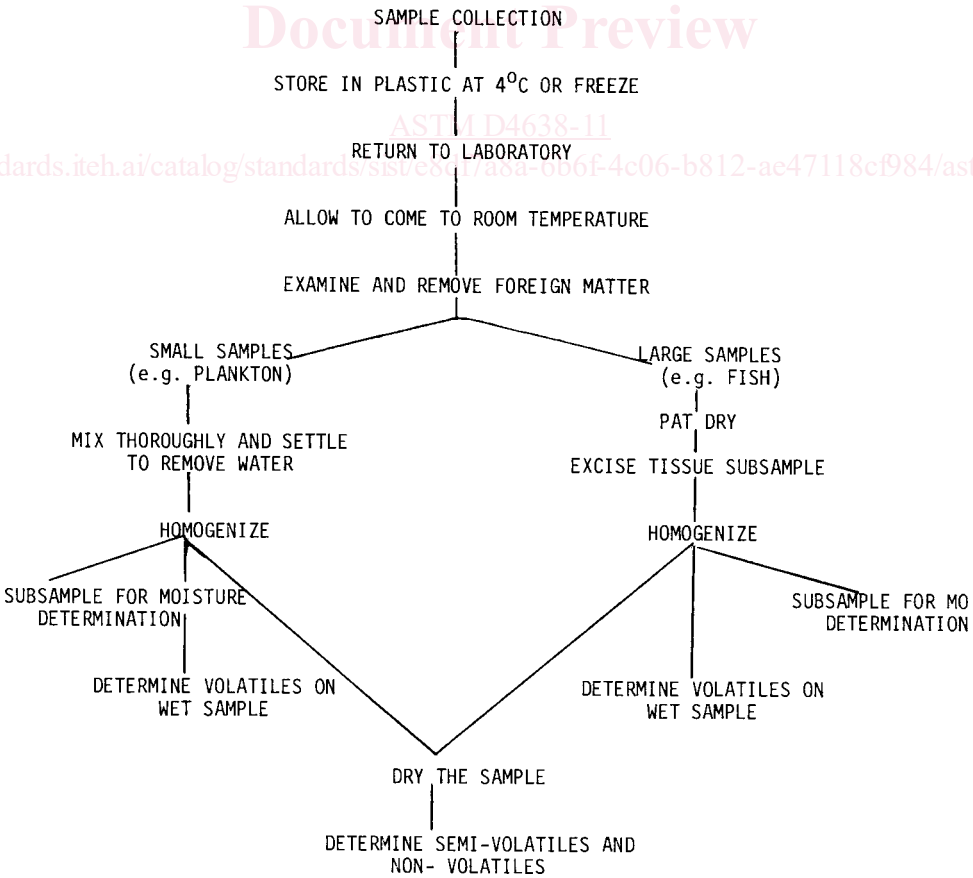

FIG. 1 Flow Diagram for the Preparation of Biological Samples for Inorganic Analysis

size sufficient for the analysis. Using a tissue disrupter, blender, or equivalent, homogenize the sample, or composite (to ensure lack of contamination, carry a standard or blank, or both, through this procedure), and proceed to Section 7.

6.2 Treat large samples such as fish as follows:

6.2.1 Allow the sample to return to room temperature.

6.2.2 Pat the sample dry with paper toweling to remove as much water as possible.

6.2.3 Transfer the sample to a nonmetallic surface, such as a flat glass plate, and excise a sufficient quantity of material, or specific organs, to obtain sufficient material for analysis. Make excisions with plastic knives or TFE-fluorocarbon-coated scalpels.

6.2.4 If chemical analyses are to be carried out on a wet sample, use a tissue disrupter, blender, or equivalent, to homogenize the material (to ensure lack of contamination, carry a standard or blank, or both, through this procedure). Remove a subsample for moisture determination and proceed to Section 7. Retain the remainder and proceed to Section 9.

6.2.5 If chemical analyses are to be carried out on a dry sample, use a tissue disrupter, blender, or equivalent, to homogenize the material (to ensure lack of contamination, carry a standard or blank, or both, through this procedure) and proceed to Section 7.

7. Drying Procedures

7.1 Use a sample or subsample prepared in accordance with the directions given in Section 6.

~~7.2Treat~~7.2 Treat subsamples from biological materials that are to undergo chemical analysis without drying for moisture determinations as follows:

7.2.1 Accurately weigh 5 to 10 g ± 1 mg or 10 to 25 g ± 10 mg of material into a nonmetallic container which has been previously tared, and weighed with the same accuracy.

7.2.2 When a limited amount of material is available, determine the moisture on a 1 to 2-g sample, and weigh with an accuracy of ±0.1 mg. The use of samples smaller than 1 g is not recommended for moisture determination.

7.3 When an entire sample is to be dried prior to chemical analysis, a moisture determination is also required. Transfer the accurately weighed material (1 to 2 g ± 0.1 mg, 5 to 10 g ± 1 mg, >10 g ± 10 mg) into a dry nonmetallic container which has been previously tared, and weigh with the same accuracy.

7.4 If a moisture determination (or sample drying) is to be made using an oven, treat as follows:

7.4.1 Transfer the containers holding the material to an oven and dry for 2 h at one of the following temperatures:

7.4.1.1 For the determination of semivolatile constituents, use the temperature specified in the analytical procedure for the constituents(s).

7.4.1.2 For determination of nonvolatile constituents use 105 ± 2°C.

7.4.2 Cool in a desiccator, then weigh the dried samples with the same accuracy as the wet samples.

NOTE 1—Biological materials tend to be very hygroscopic. Keep weighing times to a minimum.

7.4.3 Repeat drying at hourly intervals, to attain a constant weight.

7.5 If a moisture determination (or sample drying) is to be made at room temperature, treat as follows:

7.5.1 If drying is to be done in a desiccator, ensure that the desiccant in the bottom is fresh, and some means is available to indicate when the desiccant loses its drying capacity (for example, color change). A vacuum desiccator may also be used.

NOTE 2—If a vacuum desiccator is used, bear in mind that this may cause the loss of volatile or semivolatile inorganics such as mercury, if the dried sample is to be subjected to chemical analysis.

7.5.1.1 Transfer the containers holding the material to a desiccator.

7.5.1.2 Leave the material in the desiccator for 48 h, then weigh the dried samples with the same accuracy as the wet sample.

7.5.1.3 Repeat weighings at 4-h intervals, to attain a constant weight (see Note 1).

7.5.2 Alternatively, sample drying or moisture determinations may be carried out in a laminar flow hood; treat as follows:

7.5.2.1 Transfer the containers holding the material to an appropriate hood and turn it on.

7.5.2.2 Leave the material in the hood for 48 h, then weigh the dried samples with the same accuracy as the wet sample.

7.5.2.3 Repeat weighings at 4-h intervals, to attain a constant weight (see Note 1).

NOTE 3—Air-drying in the open is strongly discouraged unless it is carried out in a clean room, where possible contamination from airborne particulates can be controlled.

7.6 If a moisture determination (or sample drying) is to be made using a freeze dryer, treat the determination as follows:

7.6.1 Transfer the containers holding the material to the freeze dryer.

7.6.2 Follow the manufacturer's instructions for the particular unit in use. Make certain that a trap is placed between the vacuum pump and the drying chamber to prevent pump oil fumes from possibly contaminating the sample. Drying is usually complete when the internal pressure in the drying chamber reaches 50 millitorrs or less.

7.6.3 Transfer the freeze-dried samples to a desiccator for storage, and weigh them with the same accuracy as the wet samples (see Note 1).

NOTE 4—Because freeze drying occurs under vacuum, this may cause the loss of volatile or semivolatile inorganics such as mercury, or both, if the dried sample is to be subjected to chemical analysis.

7.7 The possibility of loss of volatile constituents dictates the drying procedure to be used, prior to chemical analysis. Determine