



Standard Practice for Evaluating an Effluent for Flavor Impairment to Fish Flesh¹

This standard is issued under the fixed designation D 3696; 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 practice describes procedures for determining the potential of an effluent to cause flavor impairment of fish flesh. Fish can be exposed to effluent either caged at contaminated sites adjacent to a discharge, or to dilutions of an effluent in a laboratory. Depending upon the uptake rate of the chemicals from the discharge into the flesh, 1 day to several weeks might be required before an off flavor is detectable. However, an exposure of 10 days is usually adequate. This practice is applicable to fish in either fresh or salt water.

1.2 *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 statements are given in Section 7.

2. Referenced Documents

2.1 ASTM Standards:

- D 1125 Test Methods for Electrical Conductivity and Resistivity of Water²
- D 1126 Test Method for Hardness in Water²
- D 1129 Terminology Relating to Water²
- D 1192 Specification for Equipment for Sampling Water and Steam²
- D 1252 Test Method for Chemical Oxygen Demand (Dichromate Oxygen Demand) of Water³
- D 1293 Test Methods for pH of Water²
- D 1888 Test Methods for Particulate and Dissolved Matter, Solids, or Residue in Water²
- D 2579 Test Methods for Total and Organic Carbon in Water³
- D 3250 Test Method for Total Oxygen Demand in Water³
- D 3370 Practices for Sampling Water²
- E 729 Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians⁴
- E 943 Terminology Relating to Biological Effects and Environmental Fate⁴

¹ This practice is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.01 on Aquatic Toxicology.

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² *Annual Book of ASTM Standards*, Vol 11.01.

³ *Annual Book of ASTM Standards*, Vol 11.02.

⁴ *Annual Book of ASTM Standards*, Vol 11.05.

E 1192 Guide for Conducting Acute Toxicity Tests on Aqueous Effluents with Fishes, Macroinvertebrates, and Amphibians⁴

E 1810 Practice for Evaluating Effects of Contaminants on Odor and Taste of Exposed Fish⁴

3. Terminology

3.1 *Definitions*—For definitions of terms used in this practice, refer to E 943.

3.2 *Description of Terms Specific to This Standard:*

3.3 *flavor impairment*—a detectable flavor deterioration between a test and control sample. Flavor tainting, off flavor, and undesirable flavor are considered synonyms.

3.4 The words “must,” “should,” “may,” “can,” and “might” have very specific meanings in this practice. “Must” is used to express an absolute requirement, that is, to state that the test ought to be designed to satisfy the specified condition, unless the purpose of the test requires a different design. “Must” is only used in connection with factors that directly relate to the acceptability of the test. “Should” is used to state that the specified condition is recommended and ought to be met if possible. Although violation of one “should” is rarely a serious matter, violation of several will often render the results questionable. Terms such as “is desirable,” “is often desirable,” and “might be desirable” are used in connection with less important factors. “May” is used to mean “is (are) allowed to,” “can” is used to mean “is (are) able to,” and “might” is used to mean “could possibly.” Thus the classic distinction between “may” and “can” is preserved, and “might” is never used as a synonym for either “may” or “can.”

4. Summary of Practice

4.1 Fish are exposed to an effluent or dilutions of an effluent either in the field or in the laboratory. After an exposure sufficient to allow tainting, fish are removed and fillets are evaluated for flavor impairment using the practice described in E 1810. Fillets are those portions of a fish exclusive of scales, bones, head and fins that are normally consumed. Only live fish should be used for the taste test.

5. Significance and Use

5.1 This methodology, although subjective, can detect contamination of a fishery resource. Varying results may be obtained depending on the selection of fish and the sensitivity of participants in the taste panel. Nevertheless, enforcement agencies usually consider water that produces an undesirable

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

flavor to fish flesh to violate water quality standards.

6. Apparatus

6.1 Field Study:

6.1.1 *Cages*—Cages should be large enough to allow free swimming of the fish. The wire mesh or holes used to provide water circulation into and out of the cage should be small enough to retain small minnows, yet large enough to allow free passage of stream drift organisms. A 5-mm screen has proven satisfactory. See Fig. 1 for a typical fish exposure cage.

6.1.2 *Nets*—Nets used to capture and handle test animals should be pocketed to retain fish. Use soft nets to prevent abrasion of the test fish during handling. Thoroughly wash new nets to remove any textile finish.

6.1.3 Chest Waders or Hip Boots.

6.1.4 *Boat*—Depending upon the nature of the study, especially when large rivers, lakes or estuaries are being investigated, a boat is necessary. The type of boat necessary must be matched to the type and size of the water body.

6.1.5 Life Preservers

6.1.6 *Holding Cages or Tankage*—Fish should be held in cages or pens placed at clean sites in the river or in aquaria of appropriate size for the fish for at least 10 days before testing. The grams of fish per liter volume of cage, pen, or aquarium should not be so high that it affects the results of the test. Therefore, the loading should be limited to ensure that (1) the concentrations of dissolved oxygen and test material do not fall below acceptable levels, (2) concentrations of metabolic products do not exceed acceptable levels, and (3) the test organisms are not stressed because of aggression or crowding. Suggested loadings for aquaria found in E 729 based on temperature regime should be applied to both cages and aquaria unless data are provided to justify some other loading.

6.2 Laboratory Study:

6.2.1 *Flow-Through System*—Many metering systems can be used. Guides E 729 and E 1192 describe suitable systems that are or can be modified for effluents.

6.2.2 *Compressed Air* (oil-free)—A low-pressure high-volume air blower system is ideal for providing air to support life in laboratory aquaria.

6.2.3 *Exposure Aquaria*—Aquaria should be large enough to provide an adequate volume of water and sufficient space for the fish being exposed.

6.2.3.1 Aquaria and other equipment that will contact stock solutions, test solutions, or any water into which test organisms will be placed should not contain substances that can be leached or dissolved by aqueous solutions in amounts that adversely affect test organisms. In addition, aquaria that contact test solutions should be chosen to minimize sorption of test materials from water. Glass, Type 316 stainless steel, nylon, and fluorocarbon plastics should be used whenever possible to minimize dissolution, leaching, and sorption, except that stainless steel should not be used in tests on metals in salt water. Concrete and rigid plastics may be used for holding and conditioning tanks and in the water-supply system, but they should be soaked, preferably in flowing dilution water, for a week or more before use (E 729). Brass, copper, lead, galvanized metal, cast-iron pipe, and natural rubber should not contact test solutions before or during the exposure. Items made of neoprene rubber and other materials not mentioned above should not be used unless it has been shown that either (a) unfed individuals of a sensitive aquatic species do not show more signs of stress, such as discoloration, unusual behavior, or death, when held for at least 96 h in static dilution water in which the item is soaking than when held in static dilution water that does not contain the item, or (b) their use will not adversely affect survival, growth or reproduction of a sensitive species.

6.3 Equipment Required for Taste Evaluation:

6.3.1 See ASTM E 1810.

7. Hazards

7.1 Follow local water safety laws and practices in field studies. Check with local enforcement agencies, since these laws vary from one area to another. When wading in water, wear boots or chest high waders. Wear a life vest or preserver when wading in deep water or in a boat.

7.2 Do not wash fish that are being cleaned in the field with effluent or the dilution water (river, lake, etc.). Use paper towels to wipe the fish clean. Do not taste fish that are dead in the exposure tank or show any signs of toxic effects, because they might be toxic to the taster or possible tissue deterioration might influence the test results.

7.3 Minimize personal contact with the effluent or dilutions of the effluent because it is always possible that some hazardous material, bacterial, or viral pathogen might be present. Thoroughly clean hands, clothing, and equipment after contact.

7.4 Chemical and microbiological analysis of effluent should be undertaken before exposing fish. Chemical analytes should include metals, volatile and semi-volatile organic compounds, pesticides, and any other chemicals known or believed to be present in the exposure medium. Use appropriate safety equipment when handling effluent and fish.

7.5 When sampling, preparing and performing odor and flavor evaluations, follow all safety precautions described in E 1810.

8. Field and Dilution Water

8.1 At a minimum, water quality for field or laboratory water in which fish are exposed for any flavor impairment tests must allow fish to survive with no obvious internal or external lesions forming. For field or dilution water used in laboratory

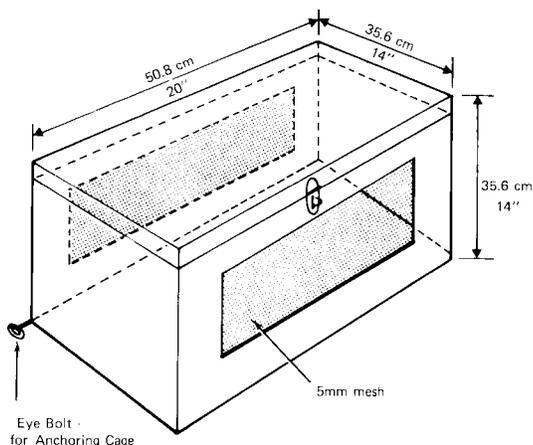


FIG. 1 Fish Cage

tests, first instar (newly hatched) daphnids must be able to survive for 48 h without food (see Guide E 729). The water must be free of taint producing materials. Sample water for chemical analysis in accordance with Specification D 1192 and Practices D 3370. Some suggested chemical analyses are given in Test Methods D 2579, D 1252, D 3250, D 1293, D 1126, D 1125, and D 1888 or use appropriate EPA methods.

9. Test Organisms (Fish)

9.1 Any edible fresh or salt water fish available in sufficient numbers is an acceptable test species. Cultured fish, such as rainbow trout, *Oncorhynchus mykiss* Richardson, and bluegill, *Lepomis macrochirus* Rafinesque, are two freshwater species that have commonly been used. Fish selected for exposure should be large enough to provide a 10 g fillet per panelist. Trout 200 to 300 mm or bluegill 150 to 200 mm are of sufficient size. In the absence of specific information relating fish length to fillet size, the user of this designation should first determine that fish of the size selected will yield fillets of the appropriate size. The size of each fish should be recorded along with data regarding age and life cycle stage. For field exposures, the season(s) during which the fish were exposed should be recorded. Gender of each exposed fish should be determined at the time that fillets are removed from the fish.

9.2 Before testing, hold fish for at least 10 days in flowing water of similar quality to the dilution water to be used for experimental exposure. Maintain a sufficient flow of water to maintain the dissolved oxygen at or above 60 % saturation and to flush out fish excretory products. Holding temperature should be $\pm 2^{\circ}\text{C}$ of the exposure temperature.

9.3 Fish should be fed an uncontaminated food during the initial quarantine and test exposure periods. Materials in food might be bioaccumulated from the food and cause flavor impairment.

9.4 Conducting fish-taste-tainting studies might require a permit of some type; therefore, notify the local health and environmental regulatory agencies.

10. Procedure

10.1 Field Exposure of Fish:

10.1.1 To test for taste and odor tainting, place fish in cages at various locations in streams, rivers or estuaries receiving discharge from an outfall. Cages should be made of wire mesh small enough to retain minnows or other items provided as food and strong enough to retain the test species. In a river or stream, place cages upstream of the outfall, at the outfall, and at one or more sites downstream. In an estuary, place cages at the outfall and at a series of locations fanning out at increasing distances from the outfall. The number of cages placed around an outfall and the distances of sites from the outfall may vary depending upon the hydrology of the water body. The average wind velocity and direction and other factors might also affect the effluent concentration to which the fish are exposed. Placement of the cages with respect to the area of study and depth is at the discretion of the investigator. An exposure of 10 days is accepted as adequate.

10.1.2 For tests in rivers and streams, place at least one cage of fish as a control in the water upstream from the outfall. For tests in estuaries, place at least one cage of fish as a control in

water remote from the outfall, but with similar salinity. If not possible within the study estuary, select a clean site in a nearby estuary with similar temperature, salinity, dissolved oxygen, and pH to the test site. Each cage or pen should be constructed and located to ensure access to natural food supplies. No effort should be made to ensure quantitatively equivalent food supplies at test and reference sites. Food supplementation with commercial food should be avoided.

10.1.3 The number of fish per cage is dependent upon the size of the test species and the number of taste panel members. Plan to provide at least a 10-g fish portion per taster per exposure concentration.

10.2 Laboratory Studies:

10.2.1 Pump a representative sample (Practices D 3370) of effluent from the discharge or storage containers through a flow-through system capable of providing a series of effluent dilutions. Include a dilution water control in the test series. Place fish in each dilution and control. See 10.1.3 for suggested number of fish per concentration. Feed the fish once a day throughout the exposure. The food should be appropriate to the species of fish and free of measurable contaminants.

10.2.2 Allow sufficient flow through the test aquaria to maintain a dissolved oxygen concentration of at least 60 % saturation. Do not aerate aquaria because the flavor test material might be volatilized from the test water.

10.2.3 Maintain the test temperature of the aquaria at $\pm 2^{\circ}\text{C}$ of the average receiving water temperature outside the effluent mixing zone. If a test temperature other than that of the receiving water is used, report the reason and temperature.

10.3 The uptake of materials by fish from water varies from one material to another. Because of this variation, no one exposure period will cover all situations. However, an exposure period of 10 days is recommended.

10.4 After exposure, remove, process and preserve fish as described in E 1810.

10.5 On the day of the flavor evaluation, prepare the fish for tasting in accordance with E 1810.

10.6 Flavor Impairment Panel:

Details of the protocols for establishing a flavor impairment panel and all aspects of performing the taste test are described in E 1810.

11. Calculation of Results

11.1 See ASTM E 1810.

12. Report

12.1 For field-collected fish, report the geographic coordinates of the collection site, a thorough description of the site, any known history of the location and adjacent land use, all available data characterizing the water and underlying sediment, the method of collection and handling in the field, list all persons involved in collecting the fish, and include the chain-of-custody form. For laboratory-contaminated fish, report the exposure protocol, the nature of the material to which the fish are exposed, the nominal and measured exposure concentrations, duration of exposure, dates of the start and end of the exposure, technical staff responsible for the exposure, and include the chain-of-custody form.

12.2 Include in the report the protocol for the taste test

performed under ASTM E 1810, all results, and statistical evaluations made. If statistical procedures beyond those recommended in ASTM E 1810 are utilized, describe these in

sufficient detail that the analysis can be duplicated from the raw data independently.

SUMMARY OF CHANGES

(1) The removal of specific instructions for the performance of the taste test and substitution with reference to a companion standard (E 1810) written specifically to be a companion to this standard.

(2) A variety of editorial changes have been made.

(3) The References section was removed because all citations had been eliminated from the narrative.

(4) Information on methodological issues related specifically to estuarine situations have been added to sections 6.1.4, 10.1.1 and 10.1.2.

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