



Designation: D 3696 – 9500

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. ~~Caged fish~~ Fish can be exposed to effluent either ~~in the effluent, caged at various contaminated sites relative adjacent to the a~~ discharge, or to dilutions of ~~the an~~ effluent in a laboratory. Depending upon the uptake rate of the chemicals ~~into the flesh from the discharge, from discharge into the flesh,~~ 1 day to several weeks might be required ~~for a detectable before an off flavor is detectable.~~ However, an exposure of 10 days is usually adequate. This practice is applicable to ~~respective 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:

¹ This practice is under the jurisdiction of ASTM Committee E-47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.01 on Aquatic Assessment and Toxicology.

Current edition approved ~~Jan. 15, 1995.~~ Oct. 10, 2000. Published ~~March 1995.~~ January 2001. Originally published as D 3696 – 78. Last previous edition D 3696 – 895.

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

- 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
- 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 ~~Terminologies D 1129 and~~ 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, cleaned removed and eviscerated, double-wrapped~~ fillets are evaluated for flavor impairment using the practice described in metal foil, E 1810. Fillets are those portions of a fish exclusive of scales, bones, head and refrigerated. Each fins that are normally consumed. Only live fish sample, including control fish, is encoded should be used for identification. ~~Fish are baked at 190°C for 20 to 30 min, then tasted by the testing panel. Flavor results are evaluated statistically to detect flavor impairment. taste test.~~

5. Significance and Use

5.1 ~~This practice, methodology, although subjective, might can detect qualitative~~ contamination of a fishery resource. E Varying results may be obtained depending on the selection of fish and the sensitivity of participants in the taste panel. Nevertheless, enforcement agencies usually recognize as a consider water quality standard that no substance shall be discharged into water that imparts produces an undesirable flavor to fish.

5.2 ~~This practice does not eliminate all bias. 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 present. finish.~~

6.1.3 *Chest Waders or Hip Boots.*

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

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.

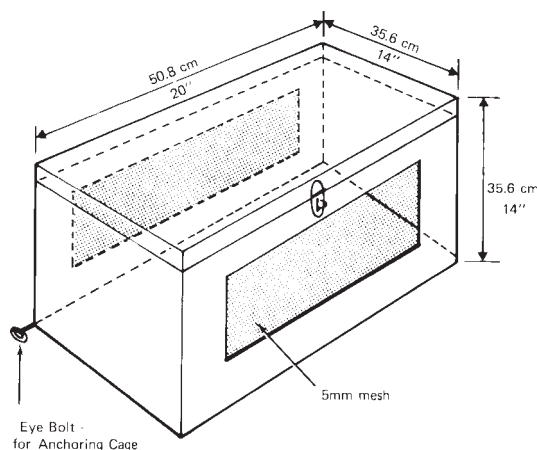


FIG. 1 Fish Cage

6.1.5 *Life Preservers*—There shall be one for each person.

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. ~~Reference (1, 2)⁵ and Guide 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 room space for the fish, such as 35 by 35 by 50 cm or larger, 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. See 7.3 Items made of Guide E 729 concerning additional 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 ~~Oven, capable of $190 \pm 5^\circ\text{C}$ (electric or gas).~~

6.3.2 ~~Plates, Glasses, and Disposal Cups.~~

6.3.3 ~~Metal Foil.~~

6.3.4 ~~Cookie Sheets.~~

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.

7.23 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.

7.3 Follow local water safety laws

7.4 ~~Chemical 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~~ 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 chest high waders. Wear a life vest or preserver when wading in deep water or in a boat.

7.4 ~~A current food handler's certificate may be required by local law for present in the cleaning, handling, 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 ~~The minimal~~

8.1 ~~At a minimum, water quality criteria for field or laboratory water in which fish are exposed for any flavor impairment testing are that the tests must allow fish will to survive with no obvious internal or external lesions forming. For field or dilution water used in it and remain healthy for the acclimation period and during the tainting tests. The more general acceptable criteria are that laboratory tests, first instar (newly hatched) daphnids will must be able to survive in the dilution water for 48 h without food (see Guide E 729). The water must be free of taint producing materials. Also sample 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.~~

8.2 ~~Fresh D 1888 or Frozen Unsweetened Lemon Juice, 1+32 dilution or weaker. 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 fair-sized fillet. 10 g fillet per panelist. Trout 200 to 300 mm or bluegill 150 to 200 mm are of sufficient size.~~

9.2 ~~Prior 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 a flow-through flowing water system of similar water quality to that of the experimental exposure dilution water to be used for at least 10 days. experimental exposure. Maintain a sufficient flow in and out of the holding tank water to provide maintain the dissolved oxygen of at least 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 stock fish initial quarantine and those being exposed, if practical, but it must be recognized that materials test exposure periods. Materials in food might be bioaccumulated from the food and also cause flavor impairment.~~

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

10. Procedure

10.1 ~~Field Studies—Using Caged Exposure of Fish:~~

10.1.1 ~~A common field technique of evaluating~~

10.1.1 ~~To test for taste and odor tainting, place fish flesh tainting can be accomplished by placing fish in cages at various locations relative to the outfall. If trout are exposed in streams, rivers or estuaries receiving discharge from an outfall. Cages should be made of wire mesh cages, include 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 above and below around an outfall and the distances of sites from the outfall may vary depending upon the hydrology of the river. In a lake or large river, 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 influence of 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, it is recommended the an exposure period be of 10 days is recommended.

10.4 After exposure, remove, kill, fillet, wrap, process and refrigerate the fish. If the flavor impairment evaluation is performed within 2 days (48 h), it is desirable to keep the preserve fish refrigerated (or iced) rather than freezing. Freezing is believed by some to alter the texture and taste of the fillet. However, if the flavor evaluation is delayed for more than 2 days, freezing the fish samples is the best method of preservation.

NOTE 1—Take special care to avoid contamination of the fish fillet with oil, gasoline, detergent, etc., because these materials impart flavor, as described in E 1810.

10.5 On the day of the flavor evaluation, ~~cut fish fillets (if frozen, thaw first) into several portions (at least 10 g) and double-wrap in metal foil with~~ prepare the same side out (shiny or dull). This might affect the cooking time. Randomly code each packet of fish to identify each individual fish, area of fish from which the fillet was taken, and the dilution or cage to which the fish was exposed. Provide enough packets of control fish for known and unknown controls, plus a few extra.

10.6 ~~Place the encoded foil packets on a flat cookie sheet and bake at 190°C for 20 to 30 min. Small portions of fish might require less cooking time; therefore, include a few extra packets of control fish to pull at earlier time intervals to check if the fish are properly baked. Overcooking dries out the flesh, seriously damaging the taste quality.~~

10.6.1 ~~An alternative method for cooking the portions is to wrap each portion tasting in a suitable polyethylene or poly(vinyl chloride) film or bag and then cook in a microwave oven for 5 min. Because various microwave ovens might vary in cooking efficiency, several extra portions should be available to check cooking times.~~

10.7 accordance with E 1810.

10.6 Flavor Impairment Panel:

10.7.1 ~~A flavor panel can be selected from associates at work, a university, or another testing group to taste the fish fillets. The panel may be comprised~~

~~Details of experienced or inexperienced fish tasters, but not a mixture of both. Inexperienced tasters would more likely represent the typical consumer. The smallest detectable concentration will probably vary depending upon whether experienced or inexperienced tasters are used. The flavor test panel should usually have at least 10 members. Flavor panels with fewer than 10 members are likely to produce results with no significant difference between the exposed fish and hidden control fish. Members of the flavor panel should be free from head colds or chest colds, and be free from allergic reactions to hayfever, and so forth, in order to perform satisfactorily as members of the flavor panel. Any physical disturbance that could affect the flavor panel at the time of taste testing should be cause protocols for preventing establishing a member from active participation at the time of testing.~~

10.7.2 ~~After the flavor panel members or consulting groups have been selected, inform them of the basic nature and purpose of the test. It may be advisable to initiate an “Informed Consent Agreement” between the flavor panel members and the sponsoring organization.~~

10.7.3 ~~Record the following information on the rating sheet (see Fig. X1.1 of the Appendix):~~

1	Name of tester
2	Date
3	Species of fish tested
4	Sample number of code
5	Rating
6	Area for comments

10.7.4 ~~After baking, rate the coded fillet samples by comparing each sample with the known control:~~

0	Same as or better than known control
1	Slight flavor impairment
2	Moderate flavor impairment
3	Strong flavor impairment
4	Extreme flavor impairment

~~Before any portion is tasted and between fish samples, rinse the mouth with dilute unsweetened lemon juice (1+32). This helps to prevent carry over of flavor from one sample to another.~~

10.7.5 ~~Prepare the impairment panel to start as soon as the fish are baked. The taste evaluation is best accomplished with as~~

little distraction as possible. Isolate panel members whenever possible while tasting. Prior to the evaluation, review the tasting and rating procedure with the panel.

~~10.7.6 Always identify at least one control fillet.~~

~~10.7.7 Before tasting begins, note the odor all aspects of performing the fish. If any odor is detected, taste samples test are described in an order of increasing odor.~~

~~10.7.8 Taste the known control first. Chew and spit out the cooked fish. Rate each fish before proceeding to the next sample. The panel member may refer back to the control as necessary.~~

~~10.7.9 For more detailed information on the principle of sensory evaluation and testing methods, consult two ASTM manuals **(3) (4).** E 1810.~~

11. Calculation of Results

~~11.1 Tabulate the results of the flavor evaluation panel, similar to that shown in the appendix. Once the data are tabulated, it can be easily seen if flavor impairment is detected. If the flavor impairment data are treated statistically, the method of analysis used should reflect the fact that data are often very consistent at high concentrations and for the hidden control, and less consistent in between (nonnormal, unequal variance). Another restriction in choosing a statistical procedure is that the ratings from each concentration are not independent because the same tasters evaluate each concentration. With these restrictions, a simple method of statistical analysis that may be used is Wilcoxon's modification of Friedman's nonparametric analysis of variance for matched samples **(5)**. This test minimizes the inherent approximation in Wilcoxon's matched-pairs sign-ranked test if a large number of ties are present, and requires only simple hand calculations. Example data are provided in the appendix (see Tables X1.1-X1.3).~~

~~11.1 See ASTM E 1810.~~

12. Report

~~12.1 A suggested listing~~

~~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 to be included characterizing the water and underlying sediment, the method of collection and handling in the report is given field, list all persons involved in Table X1.4.~~

APPENDIX

(Nonmandatory Information)

X1. EXAMPLE OF WILCOXON'S MODIFIED NONPARAMETRIC ANOVA

~~X1.1 Rank data within each taster, assigning average rank for ties.~~

~~X1.2 Calculate collecting the absolute differences between fish, and include the rank sum for chain-of-custody form. For laboratory-contaminated fish, report the control and exposure protocol, the rank sum nature of the other concentrations.~~

~~X1.3 Compare material to which the calculated results with Tables X1.1 fish are exposed, the nominal and X1.2 where: p = number measured exposure concentrations, duration of effluent concentrations exposure, dates of the start and n = number end of the exposure, technical staff responsible for the exposure, and include the chain-of-custody form.~~

~~12.42 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 calculated result is equal to or greater than analysis can be duplicated from the tabled (critical difference) result, then a significant difference exists. raw data independently.~~

REFERENCES

(1) Shumway, D. L. and Palensky, J. R., "Impairment

SUMMARY OF CHANGES

(1) ~~The removal of specific instructions for the Flavor performance of Fish by Water Pollutants," EPA-R3-73-010, National Technical Information Service, Springfield, VA, 1973, p. 80.~~

(2) ~~Mount, D. I., the taste test and Brungs, W. A., "A Simplified Dosing Apparatus for Fish Toxicological Studies," Water Research, Vol 1, 1967, pp. 21-29.~~

(3) ~~Basic Principles substitution with reference to a companion standard (E 1810) written specifically to be a companion to this standard.~~

(2) ~~A variety of Sensory Evaluation, ASTM STP 433, ASTM, 1968.~~

(4) ~~Manual editorial changes have been made.~~

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

(4) ~~Information on Sensory Testing Methods, ASTM STP 434, ASTM, 1968.~~

(5) ~~Wilcoxon, F., methodological issues related specifically to estuarine situations have been added to sections 6.1.4, 10.1.1 and Wilcox, R. A., "Some Rapid Approximate Statistical Procedures," Lederle Laboratories, Pearle River, NY, 1964. 10.1.2.~~

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