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Standard Procedure for Testing the Acute Lethality of Liquid Effluents

Canada

Regulations, Codes and Protocols
Report EPS 1-WP-80-1

Water Pollution Control Directorate
May 1982

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1 INTRODUCTION

This procedure represents a simpler alternative to the test for acute toxicity described in regulations and guidelines already published by the federal government.

While it has not been designed for scientific research, this procedure has been developed in the course of a number of years of laboratory experience, as well as through extensive review of available literature and consultation with a number of scientists. It has been demonstrated to be a valid test for lethality of an effluent, and allows a large number of tests to be performed under a variety of circumstances. Other tests may be developed in the future to accommodate changes in bioassay methods and best practicable wastewater treatment technology.

The intent of this test is to provide sufficient standardization so that the various effluents now subject to federal regulations and guidelines can be dealt with as equitably as possible. Further information regarding techniques and equipment may be obtained from the Environmental Protection Service (EPS) Laboratories listed in the Appendix.

2 PROCEDURE

2.1 Test Conditions

1) Subject to item 12, rainbow trout (Salmo gairdneri Richardson) are to be used as the test species.
2) Only healthy stocks of acclimated fish are to be used.
3) Individual fish are to weigh between 0.5 and 10 grams and the length of the largest fish should not be more than two times the smallest in the same vessel.
4) A minimum of five test fish are to be exposed to the bioassay sample for 96 hours. An equal number of control fish are to be exposed to the control water for the same time period. The test period can be terminated when more than 50 per cent of the fish exposed to the bioassay sample are dead.
5) The test is rendered invalid if less than 90 per cent of the fish in the control water survive. If only five fish are used, one mortality of the fish will render the test invalid.
6) For every one gram of fish, there should be at least 0.5 litres of bioassay sample or control water for every 24 hours that the fish are exposed.
7) The minimum water depth in any test vessel should be 15 cm.
8) If the dissolved oxygen concentration of the bioassay sample is less than 7 mg/L, the sample should be pre-aerated for not more than two hours at a rate of 5.0 to 7.5 cc/min•L prior to starting the bioassay test.
9) An aeration rate of 5.0 to 7.5 cc/min•L should be applied to the bioassay sample and control water throughout the test period.
10) The test should be conducted at 15 $\pm$ 1°C (14°C to 16°C).
11) The total number of dead fish should be observed at least once each day up to the completion of the test. Dead fish should be removed at least once each day.
12) Effluents containing only freshwater or having a salinity of less than or equal to 10 parts per thousand whether discharged into freshwater or seawater should be tested with rainbow trout acclimated to freshwater. Effluents containing seawater and/or brine and having a salinity greater than 10 parts per thousand and deposited into seawater should be tested with a fish species authorized by the Minister of the Environment and acclimated to seawater of similar salinity to that of the effluent. Effluents containing brine and

deposited into freshwater should be tested with rainbow trout acclimated to freshwater.

13) When the effluent sample must be transported or stored, the sample should be kept in sealed non-toxic containers excluding any air. The sample should not be aerated during storage, and should not be held more than five days prior to commencing the test procedure.

2.2 Interpretation of Test Results

An effluent passes this standard test for acute lethality to fish if more than 50 per cent of the fish survive the 96 hours of exposure to the bioassay sample under the standard conditions. An effluent fails the standard test for acute lethality to fish if 50 per cent or less of the test fish survive 96 hours of exposure to the bioassay sample under the standard conditions.

3 EXPLANATORY NOTES FOR THE STANDARD PROCEDURE FOR MEASUREMENT OF ACUTE LETHALITY

These explanatory notes are intended to clarify the test method described in the EPS Standard Procedure; they should not be construed as eliminating the need for applying sound judgement in the conduct of the tests. The procedure and conditions are the same as described in Standard Methods for the Examination of Water and Waste Water (13th edition, 1971) except where deviations are specifically mentioned. The most important elements of the procedure for testing lethality using rainbow trout are described.

1) Bioassay Sample

This procedure requires that an effluent sample not be diluted prior to the assay; hence, the bioassay sample is some portion of the collected effluent sample. However, some effluent regulations and/or guidelines describe dilution allowances, and they should be referred to prior to commencing the test.

2) Procurement of Fish Stocks

Stocks of healthy rainbow trout for use in these tests must be procured from one of a number of fish hatcheries approved by the Federal Department of the Environment. Collection and culture techniques for salt water organisms are varied. Advice can be obtained from the Department.

3) Holding and Acclimation of Fish

Fish should be acclimated to the laboratory conditions over a period of at least three weeks prior to testing. The purpose of acclimation is to determine that fish are healthy and to allow them to adjust to the holding conditions. The care and handling of fish is not a difficult task; however, it does require skills that come with experience. The following describes a number of conditions that are known to be important.

4) Water Quality

Water quality parameters such as hardness, pH, alkalinity, heavy metals, turbidity, chlorine, etc. are very important since they influence survival and health of fish and, if necessary, should be monitored. Chlorine, in particular, is lethal to fish in concentrations as low as 10 mg/L and occasionally lower, necessitating the dechlorination of municipal tap waters before conducting the

tests. For dechlorination, activated carbon (bone charcoal) filters are suggested. Even after contact with the carbon, residual chlorine and chloramines can remain, particularly during periods of high chlorination of tap water. In most locations these conditions occur following heavy rains or spring and fall turnover of lakes. These problems can be overcome by the use of ultraviolet radiation subsequent to activated carbon filtering (Armstrong and Scott, 1974). Ultraviolet radiation offers the added advantage of reducing bacterial levels.

5) Holding Facilities

The acclimation tanks and accessories should be made of non-toxic materials, such as glass, porcelain, fibreglass, stainless steel, polyethylene, acrylic, polypropylene or fiberglass-reinforced polyester. The acclimation tanks should be located away from any physical disturbances and preferably in a location separate from the test vessels.

During acclimation, a constant flow of water through the holding tanks is necessary. Rates of water exchange should be a minimum of 1.4 L/g of fish per day. To prevent overcrowding there should be at least one litre of water for every 10 g of fish in the holding tank (Sprague, 1973).

6) Photoperiod

The photoperiod should be a constant sequence of 14 hours of fluorescent light and 10 hours of darkness. Light intensity at the surface of the fish tanks should be 20 to 30 lux. Ideally, lights should be turned on or off gradually over at least 15 minutes, since abrupt changes in intensity startle and stress fish.

7) Aeration in Holding Tanks

Supplementary aeration should be provided if necessary to attempt to keep the dissolved oxygen concentration greater than 7 mg/L during acclimation. Supersaturation must be avoided. Filtered, oil-free compressed air is commonly used. Oxygen may be used but greater caution must then be exercised to avoid supersaturation.

8) Cleaning of Holding Tanks

Holding tanks should be kept clean. Designs for tanks which are partially self-cleaning are available. However, periodic siphoning of settled material is usually necessary. Tanks should also be disinfected with an iodophore disinfectant between batches of fish to minimize the occurrence of disease.

9) Temperature

Since the temperature of the holding water used during the transfer of fish from the hatchery to the laboratory may be outside of the acceptable limits for the test (15 ± 1°C, i.e., 14-16°C), it may be necessary to gradually change the temperature. The temperature may be changed at a rate not exceeding 5°C per day until the desired temperature for acclimation is achieved. The fish should be held at this temperature for the remainder of the 21-day acclimation period before being used in a bioassay.

10) Feeding

Feeding the fish twice each day is satisfactory. Very small fish (up to 5 cm fork length) may require additional feedings of small quantities of food every day. Fish should be fed with food containing 30 per cent to 40 per cent protein with some vegetable substances. Many commercial preparations are available. The fish should be fed an amount of food that can be consumed in about 10 minutes, approximately three to five per cent of the wet weight of the fish per day.

11) Disease Detection and Control

Daily inspection of fish in holding tanks is an essential part of the detection of disease, as "healthy" vs "unhealthy" conditions should be recognized. Recognition of the symptoms of unhealthy fish comes with experience. These symptoms include change in appetite, distribution in the holding vessel, colour and other appearances, and swimming behaviour of fish. The Handbook of Trout and Salmon Diseases (Roberts and Shephard, 1974) is a good basic guide to diagnosing and treating diseases.

12) Type of Bioassay

Bioassays applying flow-through, static, or static with replacement procedures are considered acceptable.

13) Transport, Storage and Handling of Effluent

Container(s) constructed of non-toxic materials and sealed to exclude air should be used for transportation or storage of the effluent. When possible, refrigerate the sample near 4°C. The effluent sample should not be aerated until just prior to testing.

The bioassay should commence as soon as possible after the effluent sample is collected, and should be commenced within five days of sampling.

14) Test Vessels

Test vessels should be constructed of glass or be polyethylene-lined. Liners must be rinsed with dilution water prior to use and should be used only once.

15) Definition and Sources of Control Water

Control water is the liquid that is:

a. used to acclimate the test fish;

b. used in the control part of the test; and

c. used to dilute the effluent sample, if required to form the bioassay sample.

Control water used for the above purposes should be taken from the same source. While respecting the criteria described in item 4), Water Quality (pp. 4-5), control water may be dechlorinated tap water, spring, lake, well or sea water which can be supplied with consistent quality.

16) Control Test

A test using control water should be conducted at the same time and under identical conditions as the bioassay sample. The bioassay test is declared invalid if less than 90 per cent of the control fish survive in the control vessels. For control tests using less than ten fish, a single mortality invalidates the test.

17) Start of Test

Prior to commencing the bioassay, the test vessels and accompanying tubing should be cleaned and rinsed with control water. Temperature and dissolved oxygen levels should be checked in the test vessels and the fish randomly introduced. Once all the fish have been introduced, the test commences. Pre-aeration should be minimized to avoid stripping of volatile substances.

18) Randomization

When transferring fish from the acclimation tank to test vessels some form of randomization of test vessels (e.g., using cards, dice or random tables) should be employed.

19) Observations of Mortality

The fish in the test vessels should be observed at least once each day, the number of dead fish recorded and dead fish removed. For more information, mortality can be recorded at approximately 1/4, 1/2, 1, 2, 4, 8, 24, 48, 72 and 96 hours after commencement of the bioassay. Fish are considered dead when,

upon a mild mechanical prodding, there are no visible respiratory or other movements.

20) Other Observations

Measurements should be made of the aeration rate, flow rates, temperature, pH and dissolved oxygen content of the bioassay sample and control water. These checks should be made at least daily.

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APPENDIX

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