



BIOASSAY ANALYTICAL METHODS OVERVIEW

METHOD #	METHOD TITLE	TESTING TYPE
1000.0	Fathead minnow, Pimephales promelas, larval survival and growth	Chronic Toxicity
1002.0	Daphnia, Ceriodaphnia dubia, survival and reproduction	Chronic Toxicity
2000.0	Fathead Minnow, Pimephales promela	Acute Toxicity
2002.0	Daphnia, Ceriodaphnia dubia	Acute Toxicity
2019.0	Rainbow Trout	Acute Toxicity
2021.0	Daphnia pulex and Daphnia magna	Acute Toxicity

ACUTE TOXICITY TO FRESHWATER AND MARINE ORGANISMS

These test methods consist of exposing living aquatic organisms (plants, vertebrates and invertebrates) to various concentrations of a sample of wastewater, usually from a facility's effluent stream. WET tests are used by the National Pollutant Discharge Elimination System (NPDES) permitting authority to determine whether a facility's permit and discharge complies with the WET requirements or limits.

CHRONIC TOXICITY TO FRESHWATER ORGANISMS

The tests recommend the use of the 0.5 dilution factor, five effluent concentrations and a control for six to eight days. The test methods allow for permutations such as various dilution waters, daily or three effluent sample collections for daily test renewals, and more. The biological test endpoints include survival, growth, reproduction, or teratogenicity.

SAMPLE COLLECTION REQUIREMENTS FOR WET TESTING

Sample collection quality assurance/quality control is important for WET Testing. Below are the requirements

- For toxicity testing only temperature preservation is permitted, refrigeration or icing immediately upon collection is required. Samples must be received at <6 degrees Celsius.
- The maximum hold time for WET testing samples is 36 hours. The test must be set before the 36-hour time period expires.
- A Chain of Custody (COC) form with the date and time sampling occurred is required for WET samples.

CHRONIC WET TESTING CONDITIONS

- Duration is 6-8 days for C.dubia, Is complete with 60% or more surviving control females have three broods or a maximum of 8 days.
- Duration is 168 hours +/- 2 hours for P.promelas
- Tests are conducted at 25±1°C
- Synthetic or receiving water will be used for dilution water.
- Test Concentrations: 5 for a definitive, 1 (100%) for Screens
- Test Replicates: 10 per concentration for C.dubia 4-5 per concentration for P.promelas
- Number or organisms per concentration: 10 per concentration for C.dubia and 40-50 for P.promelas
- Age of test organisms: <24 hours for C.dubia and for the P.promelas

ACUTE WET TESTING CONDITIONS

The tests in the EPA WET Acute Methods Manual consist of a control and a minimum of five effluent concentrations. The following are an overview of the testing conditions required.

- Duration is 48 hours for C.dubia and 48-96 hours for P.promelas
- Tests are conducted at 20 or 25±1°C
- Synthetic or receiving water will be used for dilution water
- Test Concentrations: 5 for a definitive, 1 (100%) for Screens
- Test Replicates: 4-5 per concentration for C.dubia and 2-5 per concentration for P. promelas
- Number or organisms per concentration: 20-50 per concentration for C.dubia and P.promelas
- Age of test organisms: <24 hours for C.dubia and 1-14 days old for the P.promelas