STATE OF WASHINGTON
DEPARTMENT OF ECOLOGY
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GUIDELINES FOR WHOLE EFFLUENT TOXICITY (WET) TESTING FOR CRUISE SHIP WASTEWATER DISCHARGES

The Memorandum of Understanding (MOU) for cruise ship discharges between the State of Washington, the Port of Seattle, and the Northwest Cruiseship Association signed April 20th, 2004 and most recently modified May 19, 2008, requires Whole Effluent Toxicity (WET) testing once every two years for cruise vessels home-ported in Washington and once every 40 port calls or turnarounds to a port in Washington for all other vessels. This requirement applies only to those vessels approved to discharge from an advanced wastewater treatment system (AWTS) continuously subject to the MOU (2.1.3). The purpose of the testing is to assess the toxicity of these effluents. The following guidelines are provided to assist the cruiseship industry and laboratories with this required testing:

1. The test facility shall be a laboratory accredited for WET testing by the State of Washington.
2. The samples taken for the WET testing shall be taken from discharges entering waters subject to the MOU.
3. Test samples should be representative of the discharge. Multiple grab samples (minimum of four grabs with a minimum of ½ hour between grabs) shall be taken from the same discharge and composited to be representative. If gray water and black water are discharged separately, then they shall be sampled and tested for toxicity separately.
4. The tests shall be conducted with a dilution series of 100%, 50%, 25%, 12.5%, 6.25% and 3.125% sample (a 0.5 dilution factor).
5. The tests shall be run at 20 degrees C.
6. Acute testing shall be conducted using Topsmelt (*Atherinops affinis*) and Mysid shrimp (*Americamysis bahia* aka *Mysidopsis bahia*). The approved test procedures are found in EPA 821-R-02-012. EPA/600/R-95-136 will need to be consulted for specifics on testing with Topsmelt.
7. The following sampling/reporting requirements shall be followed:

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a.) All reports shall be submitted in accordance with the most recent version of Department of Ecology Publication #WQ-R-95-80, *Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria*[1] in regards to format and content. Reports shall contain bench sheets and reference toxicant results for test methods. If the lab provides the toxicity test data on electronic storage media for electronic entry into the Department's database, then the discharger shall send the electronic storage media to the Department along with the test report, bench sheets, and reference toxicant results.

b.) Testing shall be conducted on grab samples (minimum of four grabs with a minimum of ½ hour between grabs). Samples shall be shipped on ice to the lab immediately upon collection. If a grab sample is received at the testing lab within one hour after collection, it must have a temperature below 20° C at receipt. If a grab sample is received at the testing lab within 4 hours after collection, it must be below 12° C at receipt. All other samples must be below 6° C at receipt. The lab shall begin the toxicity testing as soon as possible but no later than 36 hours after sampling was ended. The lab shall store all samples at 4° C in the dark from receipt until completion of the test.

c.) All samples and test solutions for toxicity testing shall have water quality measurements as specified in Department of Ecology Publication #WQ-R-95-80, *Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria* or most recent version thereof.

d.) All toxicity tests shall meet quality assurance criteria and test conditions in the most recent versions of the EPA manual listed in subsection A and the Department of Ecology Publication #WQ-R-95-80, *Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria*. If test results are determined to be invalid or anomalous by the Department, testing shall be repeated with freshly collected effluent from the next discharge event.

e.) Control water and dilution water shall be laboratory water meeting the requirements of the EPA manual or pristine natural water of sufficient quality for good control performance.

f.) The whole effluent toxicity tests shall be run on an unmodified sample of final effluent unless chlorine is used. If chlorine is used, the final effluent samples for whole effluent toxicity testing shall be chemically dechlorinated with sodium thiosulfate just prior to test initiation. No more sodium thiosulfate shall be added than is necessary to neutralize the chlorine. Calculations to determine the appropriate amount of sodium thiosulfate shall be included in the test report.

g.) All whole effluent toxicity tests that involve hypothesis testing, and do not comply with the acute statistical power standard of 29% as defined in WAC 173-205-020[2], must be repeated on a fresh sample with an increased number of replicates to increase the power.

[1]Department of Ecology Publication #WQ-R-95-80, *Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria:* http://www.ecy.wa.gov/biblio/9580.pdf

[2]Chapter 173-205-020 Washington Administrative Code (WAC): http://www.ecy.wa.gov/pubs/wac173205.pdf