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Whole Effluent Toxicity (WET) Testing with *Ceriodaphnia dubia*

Benefits of WET Testing

Whole Effluent Toxicity (WET) testing is a biological assessment method used to evaluate the toxicity of complex effluents, such as treated wastewater or industrial discharges, to aquatic organisms. Rather than analyzing individual chemical components, WET testing exposes living test organisms, such as *Ceriodaphnia dubia*, *Oncorhynchus mykiss* (rainbow trout), and *Daphnia magna*, directly to effluent samples to measure survival, reproduction, and/or growth responses.

One of the key benefits of WET testing is its ability to measure cumulative biological effects of complex effluents, including effects that may not be predicted from chemical-specific analyses alone. WET testing provides a direct biological response and can support a more complete assessment of effluent quality. It can also serve as an early warning tool, helping municipalities and industries identify toxicity issues, investigate potential causes through Toxicity Identification Evaluation (TIE) procedures, and implement corrective actions.

Advantages of *Ceriodaphnia dubia* for Chronic Toxicity Tests

Ceriodaphnia dubia is a small freshwater crustacean belonging to the order Cladocera, commonly referred to as a “water flea”. Measuring about 0.3 to 1.0 mm in length, *C. dubia* is a microscopic zooplankton organism found naturally in lakes, ponds, and slow-moving streams throughout North America. *C. dubia* feeds primarily on algae, bacteria, and other small organic particles suspended in the water column, and



Ceriodaphnia dubia

is prey to small fish and invertebrates. Its role in the aquatic food web is important, because it transfers energy from algae and other suspended food particles to higher trophic levels.

C. dubia reproduces rapidly under favourable conditions, primarily through asexual reproduction, and is highly sensitive to changes in water quality. Because of this sensitivity, it is one of the most widely used test organisms in aquatic toxicology and regulatory ecotoxicology. Environment Canada’s EPS 1/RM/21 method uses *C. dubia* to evaluate the potential chronic effects of effluents, chemicals, and other test substances on survival and reproduction. Chronic toxicity tests evaluate longer term effects such as metabolism, growth, reproduction, and survival, and use a test cycle with an exposure period that represents a significant portion of the organism’s lifespan – typically at least 10%.

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The short reproductive cycle and well-documented stress responses of *C. dubia* make it a useful biological indicator for assessing chronic effects from industrial effluents. Its small size reduces the sample volume required for testing, simplifying sampling and transportation logistics.

Canadian Regulatory Use of *Ceriodaphnia dubia* Testing

In Canada, WET testing is a key component of environmental effects monitoring (EEM) programs under Fisheries Act regulations. These programs assess whether discharged effluents may cause deleterious biological effects in receiving environments.

One example is the Metal and Diamond Mining Effluent Regulations (MDMER). The EEM requirements under the MDMER include both acute lethality testing and sub-lethal toxicity testing. Sub-lethal toxicity testing includes tests using fish, invertebrate, plant, and algal species. For freshwater invertebrate reproduction and survival testing, the required method is Environment Canada EPS 1/RM/21, *Biological Test Method: Test of Reproduction and Survival Using the Cladoceran Ceriodaphnia dubia*.

Under the MDMER, sub-lethal toxicity tests are conducted twice per calendar year for the first three years, then yearly after the third year, subject to regulatory conditions and discharge-specific requirements.

Similar *C. dubia* sub-lethal toxicity testing requirements are included within other Canadian EEM programs, such as those mandated by the federal Pulp and Paper Effluent Regulations.

Overview of *Ceriodaphnia dubia* Survival and Reproduction Test

The Environment Canada EPS 1/RM/21 toxicity test method is a 6- to 8-day test that measures the effects of an effluent or other test substance on the survival and reproduction of *C. dubia*. The test uses neonates that are less than 24 hours old at the start of the test, born within 12 hours of each other, sourced from a healthy laboratory culture.

Individual organisms are placed into separate test vessels containing a control or one of several effluent concentrations and are observed daily throughout the test period under controlled temperature and lighting conditions. Test solutions are renewed daily using a static renewal design to maintain effluent freshness and water quality. Nutrient levels are maintained to support normal growth and reproduction. A standard multi-concentration test design includes a control and a geometric series of at least seven effluent

concentrations, with a minimum of 10 replicate organisms per treatment. Water quality parameters, including pH, dissolved oxygen, temperature, hardness, and conductivity, are measured during the test to confirm that conditions remain acceptable.

The test ends when at least 60% of the control organisms have produced three broods, or after eight days, whichever occurs first. The primary endpoints are survival, measured as the proportion of organisms alive at the end of the test, and reproduction, measured as the number of live neonates produced per surviving female. These endpoints are used to calculate the LC50 (the effluent concentration causing 50% lethality), and the IC25 (the effluent concentration causing a 25% inhibition in reproduction relative to the control). These results are used to characterize effluent toxicity.

To ensure test validity, the method requires that control organisms meet minimum performance criteria. Reference toxicant testing is also conducted regularly to verify organism sensitivity and to confirm that test organisms are responding within established sensitivity ranges.

Sample Collection and Laboratory Submissions

Table 1 summarizes sample collection and submission requirements. Three 2.2 L HDPE sample containers with zero headspace are required for this test, which should be stored at 4±2°C after sampling (frozen samples are invalid). **To reserve test space in the laboratory, samples must be booked in advance with ALS in Winnipeg at least one week prior to submission.**

Ceriodaphnia dubia testing at ALS in Winnipeg is currently accredited by CALA to ISO/IEC 17025:2017 (refer to the [CALA scope of accreditation](#) for ALS in Winnipeg for present status).

Table 1. Sample Submission Details

ALS Test Code	E864
Sample Container	3 x 2.2 L HDPE Bottles Zero headspace
Transportation and Storage Temperature	4±2°C Do NOT freeze samples. Frozen samples are invalid.
Hold Time	3 days
Regular Turnaround Time	15 business days
Fastest Turnaround Time	10 business days
All samples must be booked with the lab at least one week in advance.	

References

Environment Canada. 2007. [Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*](#). Environmental Protection Series, Report EPS 1/RM/21, 2nd Edition. Environment Canada, Ottawa, Ontario.

Government of Canada. 2002. Metal and Diamond Mining Effluent Regulations, SOR/2002-222. Made under the Fisheries Act, R.S.C. 1985, c. F-14. Last amended 2026-06-04. Department of Justice Canada. <https://laws-lois.justice.gc.ca/eng/regulations/sor-2002-222>

Government of Canada. 1992. Pulp and Paper Effluent Regulations, SOR/92-269. Made under the Fisheries Act, R.S.C. 1985, c. F-14. Last amended 2026-06-04. Department of Justice Canada. <https://laws-lois.justice.gc.ca/eng/regulations/sor-92-269/FullText.html>

Please contact your ALS Project Manager for further information or to arrange for sampling supplies.

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